Faculty of Biological and Environmental Sciences
Molecular and Integrative Biosciences Research Program
University of Helsinki

Doctoral School in Health Sciences
Doctoral Programme Brain and Mind

Directional Hearing under Water: Morphology and Function of the Middle Ear of *Globicephala macrorhynchus* (Short-Finned Pilot Whale)

Itamar Tsur

DOCTORAL DISSERTATION

To be presented for public examination, with the permission of
the Faculty of Biological and Environmental Sciences of the University of Helsinki,
in lecture hall 2402, Viikinkaari 1 (Biocenter 3)
on September 24th, 2020 at 12 o'clock noon.

Helsinki 2020

Supervisors

Professor Kristian Donner
Faculty of Biological and Environmental Sciences
University of Helsinki, Finland

Docent Sirpa Nummela
Faculty of Biological and Environmental Sciences
University of Helsinki, Finland

Professor Yehudah L. Werner
Department of Ecology, Evolution and Behaviour
The Hebrew University of Jerusalem, Israel

Advisory committee

Professor Tomi Taira
Faculty of Veterinary Medicine
University of Helsinki, Finland

Professor Juha Voipio
Faculty of Biological and Environmental Sciences
University of Helsinki, Finland

Pre-examiners

Dr. Maria Morell
Institute for Terrestrial and Aquatic Wildlife Research
University of Veterinary Medicine, Hannover, Germany

Associate Professor Magnus Wahlberg
Department of Biology
University of Southern Denmark, Odense, Denmark

Opponent

Professor Peter L. Tyack
Scottish Oceans Institute
University of St. Andrews, UK

Custos

Professor Juha Voipio
Faculty of Biological and Environmental Sciences
University of Helsinki, Finland

ISBN 978-951-51-6519-0 (paperback)
ISBN 978-951-51-6520-6 (PDF)
Dissertationes Scholae Doctoralis Ad Sanitatem Investigandam Universitatis Helsinkiensis
ISSN 2342-3161 (print)
ISSN 2342-317X (online)

Cover picture: Dolphin, sculpture made by Dr. Hamish Denny, FRCVS, as a gift to IT for completing the thesis

Helsinki University Printing House
Helsinki 2020

I devote this thesis to my late elder brother, Meron Tsur. His life was a "copy-paste" of the biblical story of Iyov (Job), from which I quote the following verses (The Book of Job, Chapter one):

"1:1 There was a man in the land of Uz, whose name was Job; and that man was perfect and upright, and one that feared God, and eschewed evil.

1:8 And the LORD said unto Satan, Hast thou considered my servant Job, that there is none like him in the earth, a perfect and an upright man, one that feareth God, and escheweth evil? 1:9 Then Satan answered the LORD, and said, Doth Job fear God for nought? 1:10 Hast not thou made an hedge about him, and about his house, and about all that he hath on every side? thou hast blessed the work of his hands, and his substance is increased in the land.

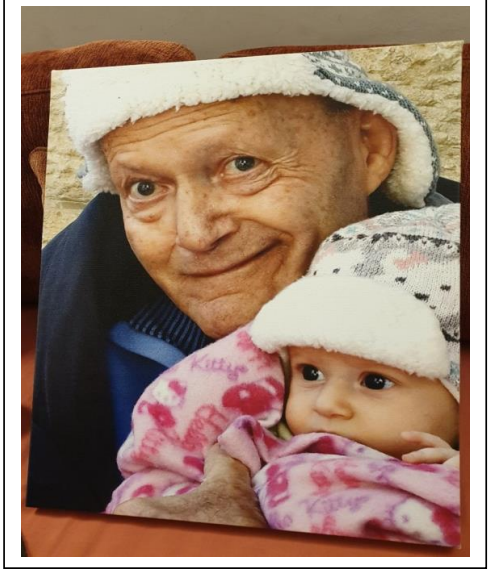
1:18 While he was yet speaking, there came also another, and said, Thy sons and thy daughters were eating and drinking wine in their eldest brother's house: 1:19 And, behold, there came a great wind from the wilderness, and smote the four corners of the house, and it fell upon the young men, and they are dead; and I only am escaped alone to tell thee. 1:20 At this, Job got up and tore his robe and shaved his head. Then he fell to the ground in worship 1:21 and said: "Naked I came from my mother's womb, and naked I will depart. The LORD gave and the LORD has taken away; may the name of the LORD be praised." 1:22 In all this, Job did not sin by charging God with wrongdoing."

Meron's mother died at his birth (1941). He was raised in an orphan's home under the British mandatory government of Palestine. His life story was a miracle akin to the story of the Baron von Münchhausen: He raised himself from physical and mental setbacks caused by the first four years of having no motherly love and caring. By his own tremendous will power, he finished high school, became an officer in the Israeli army, then completed a university degree and became a brilliant journalist and a radio broadcaster of the National Israeli Radio Services. He got married and raised a wonderful family, having a son, Ronen, and a daughter, Einat. He was a remarkably pleasant and gentle person, a knowledgeable and meticulous journalist, and a wonderfully loving husband and father. Then Satan stepped in, just like the biblical story: A stroke at the age of 55 paralyzed half his body and robbed him of his ability to speak and use his vocal instrument, upon which his livelihood depended, his wife died from brain cancer after a long struggle, his son Ronen died aged 49, from complications of heart and lung disease, and he succumbed to a fatal heart attack in December 2019. Unlike the end of the story of Job, his was not a happy one.

In all of these, he never raised a blaming finger at anyone, least of all the Powers above who inflicted all of this upon him. I want to raise my finger for him and ask:

Why?

The picture I attach herein is Meron with his youngest granddaughter, Inbar (Red headed). The smile on his face, akin only to the one which dolphins like *Tursiops* have constantly on their snout, never left him, at the hardest of times.



I am sure that our middle brother, Avner, may he live till 120, will join me in blessing Meron's soul, now that he has reached perfect and lasting Peace, free of the Pain he has endured all of his life.

Farewell Meron, you have been my Alpha dolphin all your life, and to all who have known your amazingly resilient and loving character. You have been our ever-lasting hero, and this thesis is as much about you as it is about my beloved and magnificent mammals of the Seas.

Your youngest brother Itamar

ליעל היקרה,

בתודה עמוקה על תמיכתך וסבלנותך הרבה במשך כל השנים הארוכות שראית רק את הגב שלי בזמן שכתבתי את עבודת הדוקטור, שלה היית שותפה מרכזית ואוהבת. הנה תקציר העבודה, במיוחד עבורך:

תַקצִיר

התפתחות הלווייתנים לבעלי חיים ימיים הצוללים לעומק רב לפני כ- 50 מיליון שנה גררה שינוי דרמטי בסביבתם החושית וארגון מחדש של מערכות החישה שלהם. היו לכך שתי השלכות עיקריות על מערכת השמיעה שלהם: (1) הפיזיקה של העברת קול ממים לאוזן הפנימית דרשה תכנון מחודש של המבנים המעבירים קול (אוזניים חיצוניות ותיכוניות) של אבותיהם שוכני היבשה, אשר היו מכוונים להפליא לשמיעה באוויר. (2) בסביבת הים העמוק והחשוך, השמיעה עברה את הראייה כחוש העיקרי לזיהוי אובייקטים ומיקום מקור קול במרחב התת ימי. לווייתני שיניים (אודונטוצטים) תלויים במיוחד בשמיעה מכיוון שהם משתמשים בהשמעה פעילה של צלילים בתדירות גבוהה (אקולוקיישן) כדי לאתר טרף וטורפים, ולתקשר עם בני מינם. התיזה הנוכחית עוסקת בהיבטים של שידור צליל והן בזיהוי מקור הקול במרחב התת מימי באודונטוציטים.

המטרה הראשונה הייתה להבהיר את התפקוד המכני של האוזן התיכונה כחלק משרשרת ההולכה ממבנים בראש המעבירים קול לאוזן הפנימית. הקומפלקס הטימפנו-פריאוטי (Tympano periotic) complex, TPC שונה באופן משמעותי מאוזניהם של יונקים יבשתיים. חקרנו 32 זוגות של אבר השמיעה הפריפרי TPCs של הלווייתן קצר-הסנפיר *Globicephala macrorhynchus*. זה נעשה באמצעות ויברומטריית לייזר דופלר למדידת התפלגות משרעת הרטט ב- TPC, בתגובה לרטט בתדרים שונים ששודרו לנקודה ליד היציאה של עצב השמיעה. מהתוצאות עולה כי הלוח הטימפני (Tympanic plate) פועל כמנוף המגביר את הכוח המניע תנודות בתדר גבוה (> 12 קילו הרץ) לאוזן הפנימית, דרך השרשרת של שלושת עצמימי השמע באוזן התיכונה. (מאליאוס, אינקוס וסטייפז)

המטרה השנייה הייתה להעריך האם קיימת א-סימטריה בין שתי האוזניים התיכוניות ביחס לפרמטרים קריטיים, והאם הדבר עשוי לסייע במיקום קול במרחב של עצמים הנמצאים במישור האנכי. עצמימי השמע משני הצדדים נשקלו בדיוק של מיקרוגרם, ואורכם, כמו גם שטח הבסיס של הסטייפז, נמדדו בדיוק של 10 מיקרומטר. בנוסף מדדנו את הזווית בין האינקוס לסטייפז מתמונות טומוגרפיה ממוחשבת. הבדלים משמעותיים (ערכים שמאליים> ערכים ימניים) נמצאו במשקל הגרמיות ובזווית האינקודו-סטפדיאלית. מידול תגובת המשרעת של שתי האוזניים מראה כי חוסר סימטריה אלה מביאים להבדל בתגובת האוזן התיכונה, העשוי לתמוך בלוקליזציה של מקור הקול במישור האנכי.

כאשר מייחסים את האסימטריה שנמצאה כאן ב- *Globicephala* לדיווחים קודמים על חוסר סימטריות באוזניים של יונקים וציפורים (עטלף שצד חרקים וכמה ינשופים הצדים בחושך), אנחנו מציעים שהאסימטריה מתפתחת כדי לתמוך בלוקליזציה קולית במינים הצדים בחושך במרחב תלת מימדי (בין אם באוויר או במים). כבדיקה להשערת עבודה זו, מדדנו פרמטרים של מערכת השמיעה הפריפרית אצל שני יונקים. (1) הכבשה המבויתת, כמין הקרוב לאבותיהם של הלווייתנים, (2) החתול, כטורף שצד בחושך, והפעיל בעיקר בשני מימדים, בניגוד לשלושה מימדים בהם חיים הלוויתן, העטלף והינשוף. לא מצאנו סימני חוסר סימטריה בין האוזניים התיכוניות באף אחד מהמינים הללו (חתול וכבשה).

אנו מציעים את הנחת העבודה שלנו, שאסימטריה בגרמימי השמע משותפת למינים החיים בתלת מימד, וצדים בחשיכה מוחלטת, או במקום שראייה לא יכולה לתת מענה למיקומו של הטרף בזמן אמת. היכולות האלה של מערכת השמיעה שלהם מחזקות את יכולתן לשרוד בסביבתן הטבעית.

Abstract

The evolution of whales into fully aquatic, deep-diving animals, which began over 50 million years ago, entailed a dramatic change in their sensory environment and a corresponding reorganization of their sensory systems. For hearing, this had two major consequences. (1) The physics of sound transmission from water into the inner ear required a complete redesign of the sound-transmitting structures (outer and middle ears) of their terrestrial ancestors, which were exquisitely tuned for hearing in air. (2) In the dark deep-sea environment, hearing overtook vision as the primary sense for object detection and localization at a distance. Toothed whales (Odontoceti) are crucially dependent on hearing as they use active high-frequency sounding (echolocation) to localize prey and predators and to communicate with conspecifics. The present thesis addresses aspects of both sound transmission (1) and sound localization (2) in odontocetes.

The first aim was to elucidate the mechanical functioning of the middle ear as part of the transmission chain from sound-receiving head structures to the inner ear. The odontocete tympano-periotic complex (TPC) differs significantly from temporal bone complexes of terrestrial mammals. We studied 32 pairs of formaldehyde-glutaraldehyde-fixed TPCs of the short-finned pilot whale *Globicephala macrorhynchus*. The distribution of vibration amplitudes on the TPC was measured by Laser Doppler Vibrometry while vibrations at different frequencies were applied at a point near the exit of the acoustic nerve. The results suggest that the tympanic plate acts as a lever amplifying the force driving high-frequency vibrations (> 12 kHz) into the inner ear through the ossicular chain.

The second aim was to assess whether there exists asymmetry between the left and right middle ears with respect to critical ossicular parameters, and whether this may help in localizing targets in the vertical direction. The malleus, incus and stapes on both sides were weighed with µg accuracy and their lengths as well as the stapedial footplate area measured with 10 µm accuracy. Further, the incudo-stapedial angle was measured from computer tomography images. Significant differences (left values > right values) were found in the weights of the ossicles and in the incudo-stapedial angle. Modelling the amplitude responses of the two ears shows that these asymmetries result in an elevation-dependent difference signal that may support localization of a sound source in the vertical direction.

When relating the asymmetry found in *Globicephala* to earlier reports of ear asymmetries in mammals and birds (one bat species and several owls hunting in the dark), I hypothesized that asymmetry evolves to support sound localization *in species that hunt in darkness in three-dimensional space* (whether in air or water). As a pilot test of this working hypothesis, we carefully dissected the middle ears and measured ossicular parameters with high accuracy in two mammals, where left-right asymmetry, if present, would necessarily implicate other factors. (1) The sheep, as a close relative of the immediate terrestrial ancestors of whales, might reveal if whale asymmetry depends on phylogenetic heritage. (2) The cat, as a dark hunter active mainly in two dimensions, might reveal if asymmetry is a more general property of nocturnal predators. We found no signs of asymmetry between the middle ears in either of these species.

The thesis is based on the following original works which will be referred to by their Roman numerals in the text:

I. Tsur I, Shaviv N, Bronstein I, Elmakis D, Knafo O, Werner YL (2019). Topography of vibration frequency responses on the bony tympano-periotic complex of the pilot whale *Globicephala macrorhynchus*. Hearing Research 384, 107810. https://doi.org/10.1016/j.heares.2019.107810.

II. Tsur I, Shaviv N, Werner YL (2020). Dolphin (*Globicephala macrorhynchus*) middle ear: Can ossicle asymmetry aid localizing the source of incoming sounds? Manuscript submitted to the journal Symmetry.

III. Tsur I, Christie BA (2020). Middle ear functional morphology of the domestic cat (*Felis catus*) and the domestic sheep (*Ovis aries*): A comparative study with two echolocating mammals (*Globicephala macrorhynchus* and *Tadarida brasiliensis mexicana*). Manuscript.

Author's contribution:

I…..IT: collection and preservation of the material; performing the vibration experiment with the help of JR, Peabody lab. Harvard medical school. Writing of the paper. YLW: Supervising all parts, correcting and contributing to the literature, NS: Interpreting the physical results, performing the displacement and phase analysis, including their graphic representation, and offereing conclusions. DE & OK: Setting up the stiffness experiment; IB: Interpretation of the results.

II……IT: As above. Performing all the measurements of the ossicles (weights, lengths, CT images and measurement of the incudo-stapedial angles; age analysis with the help of CL, at the time in the Danish Ministry of Fisheries, Copenhagen. Writing of the manuscript. YLW and NS: As above

III….IT: Collecting the sheep and cats heads. Harvesting the cats' ossicles. Doing all the physical measurements (weights and lengths) Writing the manuscript. BAC: Microdissection of the ossicles under the microscope, providing the detailed pictures, their labelling and comments along the paper inferring function from structure.

Table of Contents

1 Introduction

Extracting information from the environment to guide biologically meaningful behaviour is a criterial attribute of animals. This particularly implies detecting, localizing and identifying important objects, such as prey, predators, or mating partners. Well-developed sensory capacities are generally coupled to active communication, involving production of signals that convey meaning to conspecifics through a given sensory modality.

Different sensory modalities, relying on different physical signals, enable communication over different ranges and with different precision in space and time. The modalities used by mammals for communication are vision, hearing, olfaction and touch. Visual communication in mammals relies on behavioural display and is relatively short-range, requiring an unobstructed line of sight in an optically clear medium. Since mammals cannot actually produce light, it is restricted to times and places with sufficient illumination. The sense of touch generally requires close proximity, most often physical contact, between sender and receiver. The sense of olfaction, relying on chemical signals spreading slowly by diffusion and convection, can work over longer distances, but with low localization accuracy. By contrast, acoustic signals combine relatively long range and high speed with reasonably good potential for spatial localization of the source. The advantages of sound and the sense of hearing are further enhanced under water. While water severely limits the speed and range of chemical signals, acoustic signals are, on the contrary, favoured, travelling with high velocity and little attenuation over large distances. Thus the highly mobile toothed whales (odontocetes), the main subject of the present thesis, are known to generate only acoustic signals. They use their vocalizations also for sensing the environment by sounding (echolocation) in the visually challenging sea environment (Bradbury and Vehrencamp 2011; Tyack 2019). Understanding whale hearing has become a matter of urgent concern after several recent mass stranding events suspected to be due to noise pollution interfering with acoustic orientation mechanisms.

In the present thesis, I have studied the role of the middle ear in determining hearing performance in odontocetes, using the short-finned pilot whale (*Globicephala macrorhynchus*) as a model. Based on an analysis of how vibrations are transmitted through the middle ear into the inner ear housing the sensory receptors, I proceed to ask whether left/right asymmetries in critical structures can support directional hearing not only in the horizontal plane, but also in the vertical plane. Finally, I assess the occurrence of middle-ear asymmetries that may be important for directional hearing more generally by a comparative study in three species of land mammals.

2 Background and literature review

2.1 Sound transmission mechanisms in mammals

Sound is a pressure wave propagating in a medium, and the conditions for propagation depend on the physical properties of the medium. Air and water set different conditions not only for sound propagation, but also for sound reception, which requires transfer of acoustic energy from the external medium into the internal medium of the organism, in mammals ultimately into the fluid-filled inner ear. The middle ear plays a crucial role in this transfer. Mammals have originally evolved as terrestrial animals, and their hearing mechanisms are designed to work in air. Modern whales, on the other hand, are fully adapted to aquatic life. This has required profound changes in the structures that channel acoustic energy to the receptors in the essentially unchanged inner ear. Thus the outer and middle ears of (ancestral) terrestrial mammals and those of odontocetes represent endpoints of a paradigmatic evolutionary continuum, where a structure optimized for one purpose (air hearing) has been reoptimized for a largely different purpose (underwater hearing) (Wagner and Schwenk 2000; Nummela *et al*. 2004a; Nummela *et al*. 2007). As a background for understanding this transformation, I shall first review some basic physics of sound.

2.1.1 Sound properties in air and water

Sound propagation. The frequency (pitch) of a sound, denoted f (cycles per second, unit 1/s = Hz), is independent of the medium. The main acoustically relevant difference between air and water lies in how they respond to the variations in pressure (p, unit $N/m^2 = kgm/s^2m^2 = Pa$) that constitute the sound wave. Water as opposed to air has very low *compressibility*, i.e. its volume changes very little in response to pressure changes. Another way of saying the same thing is that its density (ρ, unit kg/m^3) changes very little with pressure changes. Sound propagation *velocity* (c, unit m/s) in a certain medium depends on these two variables as given by the relation:

$$c \propto (\partial p/\partial \rho)^{½} \qquad (1)$$

This implies that the velocity in water is much higher than the velocity in air ($c_{water} > c_{air}$), as a much larger pressure change (∂p) is required to produce a certain small density change ($\partial \rho$). The approximate numbers (although dependent e.g. on temperature and pressure and, in water, salinity) are $c_{air} \approx 340$ m/s and $c_{water} \approx 1500$ m/s. Observing that sound advances by one wavelength (λ) for each cycle, the relation between wavelength, velocity and frequency is

$$\lambda = c/f \qquad (2)$$

Thus, for example, the wavelength of a 1000 Hz sound is 0.34 m in air but 1.5 m in water. This is immediately relevant in two respects. First, since the distance from a sound source where the mechanical vibration of the source changes into an acoustic pressure wave is on the order of one wavelength (representing the transition zone between the “near field” and the “far field”), sensing mechanical vibrations rather than pressure waves works well over longer distances in water than in air. Second, sounds carry over longer distances in water, because attenuation is basically proportional to the number of cycles performed, and with a longer wavelength the sound travels farther on a given number of cycles. Regardless of the medium of propagation, high-frequency

(short-wavelength) sounds have shorter effective ranges, but on the other hand they provide sharper spatial information about objects e.g. for echolocation (see below). Conversely, low-frequency sounds are most useful for long-range communication (see e.g. Richardson *et al*. 1995; Geisler, 1998). The low-frequency signals primarily used by baleen whales (Mysticetes) may carry over thousands of kilometres in the sea when confined to a layer called the "sound fixing and ranging" (SOFAR) channel, where vertical energy dissipation is minimized by reflection from a temperature gradient upwards and a salinity/pressure gradient downwards (Bradbury and Vehrencamp 2011) .

Acoustic impedance. When analyzing how well sound is transmitted from one medium to another, the crucial entity is *acoustic impedance Z*, which expresses the opposition of the medium to acoustic flow. It is basically the ratio of the applied pressure to the resulting particle velocity (v, unit m/s) in the medium,

$$Z = p/v \qquad (3)$$

(unit Pa/[m/s]). Water has high and air low acoustic impedance: in water even large pressures cause only small particle velocities, whilst in air small pressures suffice to cause high particle velocities. It can be shown that an equivalent expression for Z is

$$Z = \rho c \qquad (4)$$

Using the values $\rho_{air} \approx 1.3$ kg/m^3, $\rho_{water} \approx 1000$ kg/m^3, $c_{air} \approx 340$ m/s and $c_{water} \approx 1500$ m/s, we get the specific acoustic impedances $Z_{air} \approx 440$ Pa/(m/s) and $Z_{water} \approx 1500$ kPa/(m/s), i.e. a 3400-fold difference between air and water.

For sound energy to penetrate from one medium into another, the acoustic impedances of the two media must be equal or close. The greater the difference, the more of the sound will be reflected from the interface. Since biological tissues consist largely of water, the air-tissue interface appears as an impenetrable sound barrier. The actual situation in the ear is somewhat mitigated by the fact that impedance depends not only on the specific impedance of the media, but also on geometrical factors. The actual target, the inner-ear cochlea, is a quasi-open tube, and this lowers the impedance compared with bulk water by tenfold (to ca 150 kPa/(m/s), see Hemilä *et al*. 1995). Still, the mismatch at the air-cochlea interface (the oval window) remains huge.

It is the task of the middle ear to overcome this by performing *impedance matching*. In land mammals, this implies matching from air to the more than 300-fold higher acoustic impedance of the cochlea. This requires that pressure at the oval window be increased at the expense of particle motion. Underwater, however, sound reaches the animal through a medium that has 10-fold higher impedance than the cochlea. Thus, the overall matching task in whales is the opposite: to increase particle motion at the expense of pressure at the oval window. In the following two paragraphs (2.1.2 and 2.1.3) I shall summarize the designs of the middle ear in land mammals and in odontocetes from the viewpoint of impedance matching, following the accounts given by Hemilä *et al*. (1995), Nummela *et al*. (1999a, b), and Nummela *et al*. (2007).

2.1.2 Land mammal middle ear

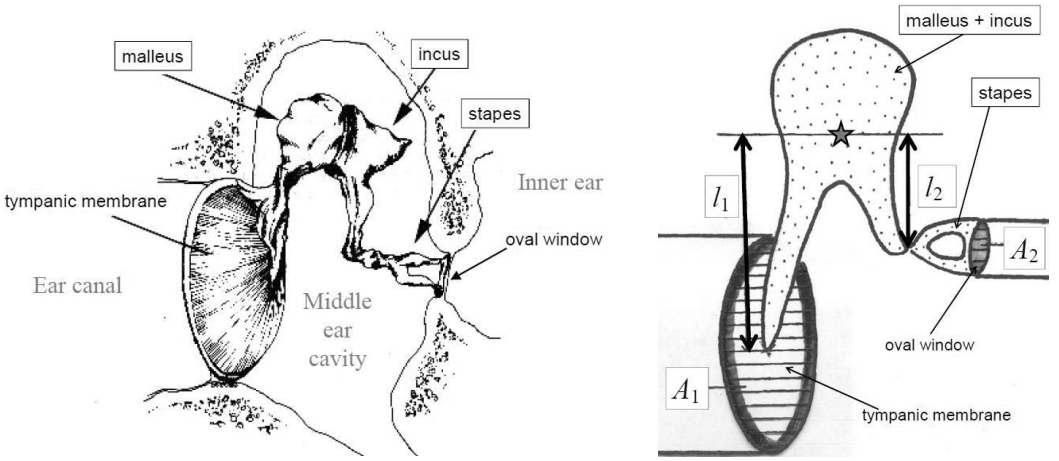
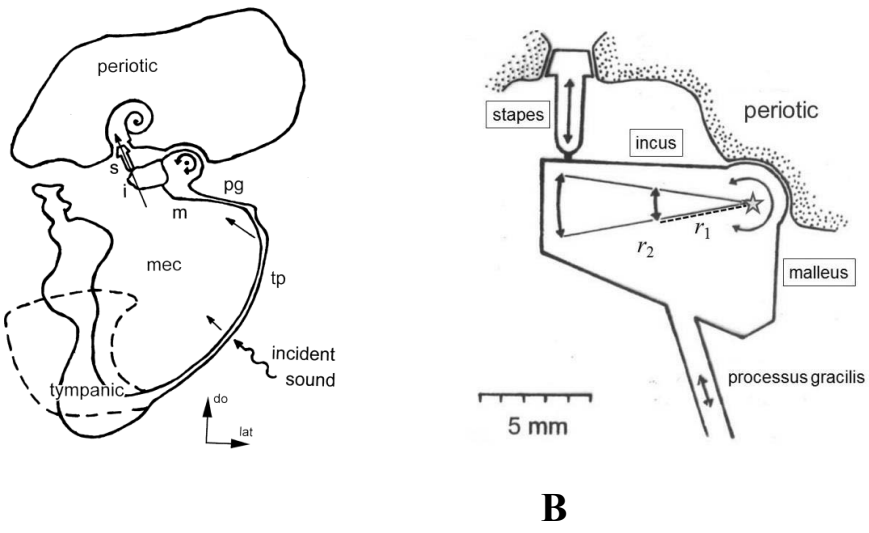


Fig. 1. A. Human middle ear (http://www.phon.ox.ac.uk/jcoleman/middle_ear.GIF). B. Schematic generalized middle ear of land mammals showing the center of rotation of the malleus-incus complex (star) and the components that produce pressure amplification: levers l_1 (malleus, set in motion by vibrations of the tympanic membrane) and l_2 (incus, moving stapes), and areas A_1 (tympanic, responding by vibrations to pressure waves in the ear canal) and A_2 (oval window, where the stapes transmits the vibrations into the inner ear). The pressure amplification from the tympanic to the oval window is (l_1/l_2) x (A_1/A_2).

In land mammals, sound is collected by the outer ear pinna and conducted by the ear canal (external auditory meatus) to the tympanic membrane, behind which lies the air-filled middle ear cavity (see Fig. 1A). As the tympanic membrane is positioned between two air-filled spaces, it responds to the incident pressure waves by mechanical vibrations. The ossicular chain of the three middle ear bones (malleus, incus and stapes) transmits the vibrations from the tympanic membrane, to which the malleus is attached by its slender manubrium, to the oval window, where the stapes footplate sets the cochlear fluid in motion. Fig 1B illustrates schematically how the impedance matching from air ($Z \approx 440$ Pa/[m/s] to the cochlear fluid ($Z \approx 150000$ Pa/[m/s]) is achieved by two pressure-amplifying mechanisms in the middle ear: (i) The malleus+incus complex, working as a single heavy lever rotating around its center of mass (star in the Figure). The lever arm (l_1) of the malleus, which contacts the tympanic membrane through a long process called the manubrium, is longer than that of the incus which contacts the stapes (l_2), hence the gain of force (and loss of velocity) from input to output is l_1/l_2. (ii) Total force is collected over the larger area A_1 of the tympanic and projected onto the smaller area A_2 of the oval window. This provides further pressure gain by the factor A_1/A_2. Obviously, the latter factor does not entail a velocity/pressure trade-off, just focussing of energy from a larger onto a smaller area. The total pressure amplification is the product of (i) and (ii), i.e. (l_1/l_2) x (A_1/A_2), which is known as the geometric transformer ratio of the middle ear.

2.1.3 Odontocete ear



A **B**

Fig. 2 (A) Semi-schematic drawing of the odontocete tympano-periotic complex based on tomography sections of killer whale middle ear at the level of the ossicles (from Hemilä *et al*. 1999, based on Nummela *et al*. 1999a). The incident sound causes vibrations of the tympanic plate (tp), which are transmitted via the processus gracilis (pg) of the malleus (m) to the ossicular chain (incus i and stapes s) and pushed into the cochlea at the oval window. mec, middle ear cavity. The straight arrows illustrate relative vibration amplitudes, increasing from the lower to the upper part of the tp and further to the stapes. The dashed contour traces the thickest part of the massive lower half of the tympanic bone, which lies in a different plane than the full-drawn structures. (B) Schematic drawing illustrating the lever ratio of the rotational movement of the fused malleus-incus complex around its center of gravity (star): r_1 is the lever on which the processus gracilis acts, r_2 is the lever acting on the stapes. The gain in particle velocity is r_2/r_1. (After Nummela *et al*. 1999b)

In modern odontocetes the outer ear has lost its function and partly disappeared. There is no pinna, which would be useless for collecting sound in water, and would be a hindrance to swimming. The ear canal is partially occluded (Ketten 1997). Instead, sound is collected by the lateral mandibular wall (Norris 1968). The mandible is lined by a fat pad with density is similar to that of sea water (Varanasi and Malins 1971, 1972), conducting the sound to the tympanic plate of the middle ear (Fig. 2A and 3B). There is a second fat body of similar density over the pan bone, a thin ovoid region in the posterior third of the mandible. The two channels, running at right angles to each other, may function as an analogue of the outer ear pinnae of land mammals, as complex interaction between the sound patterns received via the two channels on each side of the head may provide cues for vertical localization (see below). While the mandibular route is agreed to be the most important at least for high-frequency hearing (Bullock *et al*. 1968; McCormick *et al*. 1970; Møhl *et al*. 1999), the head region close to the external auditory meatus may contribute to hearing lower-frequency sounds (Bullock *et al*. 1968; Popov and Supin 1990; Supin *et al*. 2001). Indeed, there are several current hypotheses about routes by which sound can reach the inner ear in odontocetes, reviewed in paper (I).

The conceptual background of my work (papers I and II) is the account of Hemilä, Nummela and Reuter of sound transmission through the odontocete middle ear, and specifically their "four-bone model" (Hemilä *et al.* 1999; Nummela *et al.* 1999a; Nummela *et al.* 2007). The model involves four rigid bone units (the tympanic bone, the malleus-incus complex, the stapes, and the periotic bone, see Fig. 2) connected by elastic junctions. Functionally, it implements an increase of particle velocity v at the expense of pressure p by lever ratios inverted compared with land mammals. Fig. 2 A is a semi-schematic drawing from Hemilä *et al*. (1999) based on tomography sections through the killer-whale middle ear (Nummela *et al*. 1999) in planes where the ossicles are clearly visible. Fig. 2 B illustrates the movements of the ossicles and the ossicular levers involved according to the model. The incident sound sets up vibrations on the thin tympanic plate (panel A), which through the processus gracilis of the malleus causes rotation of the fused malleus+incus around the axis marked by a star in panel B. The input lever (r_1) on which the processus gracilis works is shorter than the output lever (r_2) working on the stapes, which increases particle velocity at the oval window by the factor r_2/r_1.

The full velocity amplification as defined by Hemilä et al. (1999) is the ratio of velocity at the oval window to particle velocity in the incident sound wave at the tympanic plate. The tympanic plate itself gives an important contribution to this, as its vibration amplitude in the thin part contacting the malleus is larger than its average vibration amplitude, due to the inertia of the massive edge of the tympanic bone on the opposite side. Thus the full velocity amplification is achieved by two lever mechanisms in series, the first based on the tympanic plate and the second on the ossicular chain. This provides the necessary matching from the high impedance of water to the lower impedance of the cochlea. On the other hand, if a significant part of the force collected over the large tympanic plate is projected onto the small area of the oval window, the pressure is also high at the input to the cochlea. This will directly benefit the *sensitivity* of hearing. In this respect, it is worth observing that a primary intensity amplification is achieved already when the energy collected by he large mandibular fat pad is projected on the smaller tympanic plate.

These differences in design of the hearing apparatus of odontocetes compared with land mammals are illustrated schematically in Fig. 3, based on Nummela *et al*. (2004a) as reproduced in Nummela *et al*. (2007). In addition to the odontocete features already mentioned, note the following. Air sinuses (Sin) isolate the middle ear from the skull (Sk) and effectively prevent bone conduction (see 2.1.4 below). A non-functional rudiment of the tympanic membrane persists as a ligament attached to the malleus by its medial tip. The anatomy of the tympanic bone (TyBo) differs as the medial wall in odontocetes is a thick bulky structure, the involucrum (Inv). The whole tympano-periotic complex is massive with a density as high as 2.7 g/cm^3 (Giraud-Sauveur 1969; Lees *et al*. 1983; Nummela *et al.* 1999b), which in combination with the thin bony ridges connecting the tympanic and the periotic makes the movement of the tympanic plate approximately rotational.

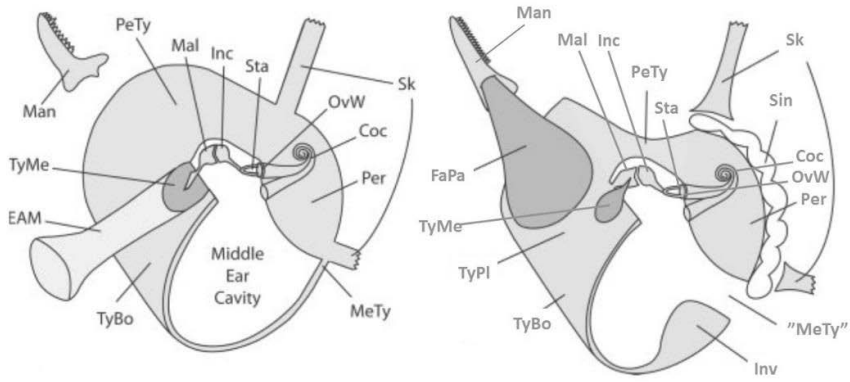


Fig. 3. Schematic comparison of land mammal (left) and modern odontocete ears (right). For the sake of visibility, the odontocete mandibular foramen and fat pad (FaPa) are shown on the lateral side of the mandible, although in reality situated on the medial side. *Abbreviations*: Coc, cochlea; OvW, oval window; Mal, malleus; Inc, incus; Sta, stapes; Man, mandible; EAM, external acoustic meatus; Inv, involucrum; Per, periotic bone; PeTy, joint between periotic and tympanic; Sin, air sinuses; Sk, skull; TyBo, tympanic bone; TyMe, tympanic membrane (rudimentary in cetaceans); TyPl, tympanic plate; MeTy, medial synostosis between periotic and tympanic bone; in cetaceans this synostosis is absent and is homologous to a gap between these bones (‘‘MeTy’’). Adapted from Nummela *et al*. (2004a) by permission from MacMillan Publishers Ltd: Nature.

2.1.4 Hearing through bone conduction

Hearing through bone conduction occurs when sound is transferred to the cochlea through vibrations of the soft tissues and bony parts of the head, not via the ear canal or the mandibular fat pad. This depends on different anatomical components in different species (Tonndorf 1968) and may physically involve either differential compression across the cochlea or differential movement of the ossicular chain and skull due to ossicular inertia, both leading to displacement of the basilar membrane. Sounds propagating in air cannot penetrate the (water-dominated) body tissues, so in land mammals most sounds carried by bone conduction represent unwanted noise from internal sources: chewing, blood circulation, breathing. (As a possible exception Reuter et al. (1998) have suggested that elephants might sense long-range, low-frequency ground vibrations by hearing through ossicular inertia). In line with this, the middle ear of terrestrial mammals is largely although not perfectly isolated from the skull, and inertial effects of the ossicles are generally minimized by having the center of gravity coincide with the rotational axis of the ossicular chain (Barany 1938). By contrast, sounds propagating in water can freely penetrate the body tissues, and bone conduction can mediate real information about the environment. In water most animals do use this hearing mechanism at least to some extent. The drawback of bone conduction is that it does not support directional hearing, as there are neither interaural differences nor direction-sensitive spectral filtering (see below). It is telling that odontocetes, which rely on sophisticated use of directional sound, have their ears effectively isolated from the skull by air sinuses (Fig. 3), thus minimizing effects of bone conduction.

2.2 Directional hearing and echolocation

Vision and hearing are the two sensory modalities where the properties of the physical signals, light and sound, in principle enable precise and fast localization even of distant objects. Taking advantage of this has been an essential success factor in the evolution of all actively mobile animals. Whenever light is scarce, hearing takes on special significance. This especially concerns warm-blooded animals active at night or in generally dark environments, e.g., in deep or murky waters. Accordingly, many such species - with owls, bats and whales as outstanding examples - have evolved amazing abilities to localize the source of a sound. Regarding mammals in general it may be said that their early evolution as crepuscular or nocturnal animals favoured refinement of other senses than vision – smell and touch and, for precise localization and communication at a distance, hearing (Grothe et al. 2010).

2.2.1 Sound localization in the horizontal plane

The dominant mechanisms for sound localization are based on comparing signals from the two ears. Only if sounds arrive in the median sagittal plane, whether from straight in front, back, above or below, will the signals arriving at both ears in principle be equal. (Mechanisms for localization in this plane will be treated together with mechanisms for vertical localization below.) Any deflection of the source to either side will cause interaural differences carrying directional information. First, the intensity of a sound will be higher at the ear closer to the source (interaural intensity difference IID), second, a sound will arrive earlier at the ear closer to the source (interaural time difference, ITD), and third, a sound will be phase-advanced at the ear closer to the source (interaural phase difference IPD).

Central processing of phase shifts (IPD) cannot be separated from the central processing of time differences and will be considered below in that context. However, it is worth mentioning that some animals may use a peripheral IPD-based mechanism. If there exists an interaural air route mediating pressure varations from one ear to the space behind the tympanum of the other ear, frequency-shifted pressure waves impinging from opposite sides will generate interference signals on the tympani. These will depend on frequency in complex manners, but may provide cues especially for animals too small for centrally detectable IIDs or ITDs to arise over the short distance between the two ears (lizards: Christensen-Dalsgaard (2005), birds: Lewis and Cole (1980), insects: Autrum (1940)). In mammals, however, the Eustachian tubes that potentially provide an air route between the two middle ears via the mouth cavity are too narrow to support interaural pressure transfer.

The fundamental binaural directional signals are IID and ITD (Masterton *et al*. 1969; Heffner and Heffner 2016). They work in the (egocentric) horizontal plane, but classical studies on the barn owl (Knudsen and Konishi 1979; Konishi 1993) have shown how asymmetry of the two ears can also provide binaural localization information orthogonal to this. Localization by intensity and time differences have their own preferential working ranges, as proposed already in 1907 by Lord Rayleigh's "duplex theory". The usefulness of ITD analysis based on phase shifts is limited to sounds with wavelengths on the order of the difference of the path lengths from the source to each ear, which is largest at 90° angle to the head, and decreases to zero when the source is straight in front of or straight behind the animal.

In water the conditions are more demanding due to the five times longer wavelength associated with any given frequency. Heffner and Masterton (1990) emphasize that the narrower the head, the higher must be the sound frequencies for this cue to work. Conversely, the higher the frequency an animal can

hear, the more likely it is to have good sensitivity for phase cues at the upper end of its frequency range (Ketten 2000). However, there is a limit when a head becomes so small that the time difference between the two ears falls below what the central nervous system can detect, no matter how high the sound frequencies, and ITD cannot be used even if the animal is perfectly able to hear those frequencies. Then IID becomes the dominant directional signal, although there is a considerable overlap of the working ranges of the two mechanisms. IIDs are also best discriminated for high-frequency sounds.

Even given the high velocity and long wavelengths of sound in water, the high frequencies that odontocetes use for signalling (from tens to more than 100 kHz) have wavelengths short enough to produce detectable ITDs across their large heads. Bottlenose dolphins studied in behavioural experiments have been found to have very accurate directional hearing (Renaud and Popper 1975). The minimum audible angle (MAA, taken as 70% correct in a 2AFC test) in the horizontal plane was 0.9° for trains of 35 μs clicks (centered on 64 kHz and presented every 3 ms for half a second). This is a prerequisite for the dolphins' ability to echolocate (see below). For longer sinusoidal pulses at 20 - 90 kHz, relevant for their communication by tonal whistles, the MAA was 2° - 3°.

2.2.2 Vertical localization

In the absence of ITDs or IIDs, sound directions can be resolved by spectral cues. Direction-dependent frequency filtering by head structures, in land mammals especially by outer ear pinnae, make signals "sound" different depending on the direction they come from. We can easily tell whether a familiar sound originates in front of us or behind us. Spectral modification as function of sound direction is called the Head Related Transfer Function (HRTF) and can contribute to localization in any direction. In land mammals it is the main mechanism for determination of the elevation of a source. Moreover, by moving its head or external pinnae a land mammal can manipulate the HRTF (as well as ITD and IID) to improve directional resolution. Spectral cues work even in purely monaural hearing (Butler 1999). For spectral filtering to be informative, however, the sound must have a complex spectrum (e.g. clicks or noise as opposed to pure tones), and must contain high enough frequencies. This is another reason why good high-frequency sensitivity is advantageous for good directional hearing.

Odontocetes have no external pinnae, but the presence of at least two separate sound pathways to each ear may provide directional information through the HRTF in analogous manner (Norris 1968; Brill *et al*. 1988; Ketten 1997, 2000; Aroyan 2001). Another factor that has to be considered particularly in odontocetes is the asymmetry of head anatomy (see 2.2.4 below). A detailed HRTF has so far been measured for only one species, the bottlenose dolphin, *Tursiops truncatus* (Taylor 2013). The results concur with several lines of evidence suggesting that odontocetes possess a complex HRTF that can support information about sound direction (behavioural experiments: Brill *et al*. (2000), electrophysiological experiments: Supin and Popov (1993), computer modelling: Aroyan (2001); see Mooney *et al*. (2012)).

While the mechanisms are thus only partly understood, the dolphins' ability to localize sounds in the vertical plane is remarkable, within measurement error equally good as in the horizontal plane. In the study of Renaud and Popper (1975) cited above, the minimum audible angle (MAA) in the

vertical direction (stimuli presented from above and below the animal's head) was 0.7° for the click stimuli and 2° - 3° for the longer sinusoids at 20 – 90 kHz.

2.2.3 Echolocation

Echolocation entails active sensing of the environment by emitting sounds and analysing the returning echoes. This capacity must be based on coevolution of sound production and reception (Au *et al*. 2009). Testifying to the value of echolocation, it has evolved independently in two completely different groups of mammalian predators active in conditions where vision is too slow, noisy or insensitive to be useful: bats and odontocetes. The amount of information that a bat can extract from the echoes, and the brain specializations that make this possible, are truly amazing, but rely on several factors not available to odontocetes: e.g., large and complex external pinnae and high-resolution analysis of Doppler effects dependent on high movement velocities relative to the speed of sound in air (Vater and Kössl 2004).

Both groups use high-frequency sounds, which, as already mentioned, enable the highest acuity regardless of localization mechanism. Being mammals with high metabolism and carrying an air reserve under water, odontocetes are able to produce very strong air-powered sounds, which are sharply projected forward by acoustic lenses and reflectors in the head (see 2.4.3 below). Echolocation clicks of the bottlenose dolphin have a rich frequency spectrum extending up to even 120 kHz. When recorded in the centre of the sound beam, clicks are much higher in intensity and peak frequency than when recorded at off-axis angles (Au *et al*. 2012). Even so, sounding exposes dolphins to predation risk from another odontocete with good high-frequency hearing, the killer whale. The evolutionary arms race has pressed the signalling of four odontocete taxa to even 150 kHz and above, beyond the hearing range of the predator (Madsen *et al*. 2005; Morisaka and Connor 2007).

Thus both localization acuity and avoidance of predatory eavesdropping require that sound emission and hearing be strongly directional and aligned (Au and Moore 1984). On the other hand, odontocetes also use click sounds, as well as tonal whistles, specifically for communication, and especially killer whales can modulate the clicks to produce complex, individuated communication signals. Many species have two sound sources; one specialized for echolocation and the other for communication. Dolphins, which communicate largely with tonal whistles, produce these from the left sound source and clicks from the right sound source (Tyack 2019).

The acoustic properties of head tissues are important for understanding odontocete sound emission and directionality. Dong *et al*. (2017) reconstructed the distribution of acoustic properties in a freshly dead specimen of short-finned pilot whale based on computed tomography (CT) and ultrasound. They determined sound velocity as well as tissue density and acoustic impedance from CT attenuation coefficients (Hounslow CT numbers). The potential of the melon to function as an acoustical lens is evident from the low sound velocity and low density of its inner core. The increase in acoustic impedance of forehead tissues from inner core to outer layer may be important for the acoustic impedance matching between the outer layer tissue and seawater. The authors also studied the temperature-dependence of sound velocity in the soft tissues. Taken together, the results provide a firm basis for modelling sound emission in *Globicephala*.

2.2.4 Head asymmetries and directional hearing

Cues for directional discrimination especially in the vertical plane can be significantly enhanced by asymmetries in skull morphology or other head structures. Among owls, which can rely entirely on hearing for prey localization in low light, bilateral asymmetry of the external ears is thought to have arisen independently in at least five lines (Norberg 1978). While the critical structures vary, the essential feature is that one ear effectively lies higher than the other, providing elevation-dependent IIDs (Payne 1971) as well as differences in spatial filtering (HRTF). Norberg (1977) emphasizes that bilateral ear asymmetry in owls involves only the external ear: "in no case has it been reported to extend into the middle or the inner ear" (cf. paper III). In behavioural experiments in near darkness, barn owls (*Tyto alba*) have been found to localize "prey" sounds (<10 kHz) with high but frequency-dependent accuracy. For this they use all the main directional cues available: IID and differential spectral filtering for both the vertical and horizontal planes (Knudsen *et al*. 1979; Knudsen and Konishi 1979) and, as shown electrophysiologically, ITD in the horizontal plane (Carr and Konishi 1990).

Mammals generally have bilaterally symmetrical skulls. This is true of the artiodactyls, the land mammals most closely related to whales, and also of baleen whales (Mysticeti). Odontocetes are exceptional in typically having asymmetrical crania, with dorsal bones shifted posteriorly and to the left (Ness 1967; Fahlke *et al.* 2011). Head asymmetries include soft tissue and cranial air sacs (Cranford *et al.* 1996, 2008; Houser *et al*. 2004). Odontocetes also have specializations for producing and projecting powerful high-frequency sounds (> 20 kHz): phonic lips, nasal sacs and a hypertrophied melon. The combination of high-frequency echolocation and cranial asymmetry, neither of which is present in mysticetes, naturally suggests that these features coevolved in the odontocete line (Mead 1975; Heyning 1989). Based on the fossil record, however, Fahlke et al. (2011) argue that asymmetry evolved in archaeocetes, before echolocation, maybe to support localization of high-frequency sounds produced by schools of fish on which they preyed, and was secondarily reduced in mysticetes as these shifted to bulk-straining predation and low-frequency hearing. By contrast, the ancestral asymmetry was enhanced in odontocetes in conjunction with high-frequency echolocation. The authors further argue that hearing-driven skull asymmetry preceded not only echolocation, but also another transition, which has been advanced as an alternative explanation: that from chewing to swallowing whole prey (MacLeod et al. 2007). Be that as it may, there is little doubt that the head asymmetries of living odontocetes can support directional hearing by position-dependent spectral filtering (Branstetter and Mercado 2006).

2.3 The impact of the middle ear on audiograms

The major success of the "four-bone" model of Hemilä *et al*. (1999, 2001) (see 2.1.3 above) is that it can provide good fits to the audiograms (hearing threshold versus sound frequency functions) of several odontocete species for frequencies up to about 100-120 kHz. One implication of this is that over the fitting range, the frequency response of the auditory system appears as largely determined

by middle-ear properties. Importantly, this further implies that modifying middle-ear parameters will have a direct impact on the frequency response of the ear.

The main parameters of the four-bone model, apart from those considered earlier (2.1.3), are the masses *m* of the tympanic bone, the malleus-incus complex, the stapes and the periotic bone, plus the spring constants *k* and damping factors *c* of five elastic couplings. The adjustable parameters *k* and *c* could not be measured by the authors and were left free for fitting, although under some constraints. The first two papers of the present thesis take this model as a framework for interpretations in *Globicephala macrorhynchus*. In paper I, I study mechanical properties of the tympano-periotic complex. In paper II, I investigate possible left-right asymmetries by measuring the masses of the malleus-incus complex and the stapes, and in addition the angle between the incus and the stapes.

In their previous study on the middle ears of land mammals, Hemilä *et al*. (1995) showed that if the high frequency hearing limit of isometric ears is limited by ossicle inertia, it should be inversely proportional to the cubic root of the ossicular mass. Ossicular mass was represented by the combined mass of the malleus + incus (the isometric stapes was left out for technical reasons, as it would not affect this proportionality relation):

$$\text{high-frequency limit} \propto 1/\sqrt[3]{(\text{malleus + incus mass})} \qquad (5)$$

This was shown by the authors to predict the high-frequency limit of 26 land mammals with a few conspicuous deviations, and thus approximately applies to the mammals in paper III. With a minor modification of how the high-frequency limit is defined, the same relation also provides a fair first-order approximation for several odontocete species (Hemilä *et al*. 2001).

2.4 Biology of *Globicephala*

2.4.1 Taxonomy and naming

Living pilot whales are classified into two species: the short-finned pilot whale (*Globicephala macrorhynchus*) and the long-finned pilot whale (*Globicephala melas*). The short-finned pilot whale was originally described based on only skeletal materials by John Edward Gray in 1846, who was led to presume that the animal had a large beak. The long-finned pilot whale was first described by Thomas Stewart Traill in 1809 as *Delphinus melas*, later renamed *Globicephala melena* and finally in 1986 regaining its original species name *melas*. Other classifications have been proposed but only these have been accepted. Genetically isolated geographic forms of short-finned pilot whales live off the east coast of Japan. Fossils of extinct relatives have been found in Pleistocene deposits in Florida, USA (*G. baereckeii*), and in Pliocene strata in Tuscany, Italy (*G. eturia*).

2.4.2 Natural history of G. macrorhynchus

Pilot whales are among the largest of the oceanic dolphins, exceeded in size only by the killer whale. Short-finned pilot whales live in the tropical and warm temperate waters of the central Atlantic, Pacific, and Indian Oceans. Males become 5.4 m long and females 4 m. They can live for

up to 50 years or more. Males reach sexual maturity in ten years, females in nine. Gestation lasts 15-16 months and calves are nursed for up to 22 months. Lactation rarely overlaps with pregnancy, and the overall reproductive cycle lasts an average of 40 months (Ridgway and Harrison 1999). A female produces up to nine calves during her lifetime. Short-finned pilot whales are among the few mammals where females go through menopause, living up to 30 years after the birth of the last calf. The societies show many matrilinear features. Studies suggest that both males and females remain in their mothers' pods, where post-reproductive females may contribute to the survival of younger members. They are highly social. Photo-identification studies in Macaronesia (the Canary Islands, Azores, and Madeira) indicate long-term relationships maintained over hundreds of kilometers and a wide variety of site fidelity patterns (Boran and Heimlich 2019). Mixing between core residents and visiting transients in high-productivity areas suggest more fluid interactions than observed e.g. in killer whales.

2.4.3 Foraging dives and vocalizations

Short-finned pilot whales have been called "cheetahs of the deep sea" (Aguilar de Soto *et al*. 2008). They are primarily squid eaters, but will feed on fish as well. Their foraging and vocalization behaviour has been elucidated in a series of studies of 23 individuals off the coast of Tenerife. Foraging dives could range below 1000 m and last over 20 min. Vocal behaviour during dives was consistent with biosonar-based foraging, with long series of echolocation clicks interspersed with buzzes such as have been associated with prey capture attempts in other echolocating species (cf. Wisniewska *et al*. 2014). In most of the deep (0.5-1 km) daytime dives, a downward directed sprint reaching up to 9 m/s occurred just prior to a buzz and coincided with the deepest point in the dive. This suggests targetting of valuable, fast-moving prey such as giant squid (Aguilar de Soto *et al*. 2008).

The economy of sound production during dives has been estimated by Foskolos *et al*. (2019) based on data from the same 23 whales. Since air supplies compress with increasing depth, deep-diving whales must use very small air volumes per click to afford sufficiently dense sampling of the environment during the foraging dives. The authors found that click production requires only 50 µL of air/click at 500 m depth, increasing gradually to 100 µL at 1000 m. With such small air volumes, the metabolic cost of sound production is on the order of 40 J per dive, which is a negligible fraction of the field metabolic rate. Still, the whales have to make frequent pauses in echolocation to recycle air between nasal sacs.

The short-finned pilot whale is a highly social species, where individuals socialize at the surface but leave their social group in pursuit of prey at depths of up to 1000 m. While diving for food, they try to retain contact with their fellow group who stayed above. While the frequency content of calls was constant, mean call output and duration decreased with depth, presumably reflecting the increasing cost of calling at greater depths (Jensen *et al*. 2011).

2.4.4 Audiograms

Audiograms of short-finned pilot whales have been determined based on recordings of auditory evoked potentials (AEP) by Schlundt *et al*. (2011) and Greenhow *et al*. (2014). In the former study, a healthy ca. 30-year old female (from Sea World in San Diego) tested with sinusoidal amplitude-modulated stimuli showed maximal sensitivity at 40-60 kHz and a high-frequency limit around 80-

100 kHz. The latter value is significantly lower than the limit measured in the bottlenose dolphin (120-150 kHz, Popov *et al*. (2010)). Interestingly, a stranded rehabilitated juvenile male showed severe loss of higher-frequency, as no AEP could be recorded in response to clicks and a response to sinusoidal stimuli was obtained only at 10 kHz. Yet the threshold at 10 kHz was the same as in the healthy female. The study of Greenhow *et al*. (2014) on four females stranded and rehabilitated in Florida confirms these results, setting the sensitivity maximum at ca. 40 kHz and the high-frequency limit around 100 kHz, depending on age: of the four females tested, the two juveniles had 25-61 dB higher sensitivity than the two adults at 80 kHz. Pacini *et al*. (2010) published the audiogram of a rehabilitated 2-year old male long-finned pilot whale showing characteristics intermediate to those of the adult and juvenile whales of Greenhow *et al*. (2014). Under the size-scaling rule given by eqn. (5), the high-frequency limits and the entire audiograms of pilot whales appear as typical among the species of odontocetes considered by Hemilä *et al*. (2001), where only the low-frequency sensitivity of the harbour porpoise and, possibly, the high-frequency sensitivity of the bottlenose dolphin appear as deviant.

2.4.5 Anthropogenic threats: noise and fishing

The more we learn of the diverse ways in which odontocetes rely on sound to solve ecological and social problems, the clearer it becomes how noise from human activities may disrupt their lives (Tyack 2009). Thus understanding how they use and respond to sound has clear practical implications for their conservation (Wartzok *et al*. 2005). Jensen et al. (2009) have shown that the noise even from small vessels at 50 m distance can reduce the communication range of pilot whales in a deep-water habitat by more than half. Cryptic odontocete species appear to react to particularly low levels of sound. For example, harbor porpoises move away more than 20 km from the sound of pile driving (Tougaard *et al*. 2009, 2014). The most intense acute responses to anthropogenic noise are mass strandings. In connection with naval sonar exercises, beaked whales stranded over tens of kilometers during a few hours (D'Amico *et al*. 2009). Pilot whales are notoriously among the most common stranders. Even if lethal stranding could be prevented, disturbances that cause whales to leave preferred habitats could adversely affect the populations (New *et al*. 2013). Short-finned pilot whales as well as false killer whales (*Pseudorca crassidens*) are also known to interact with long-line fishing gear in Hawaiian waters, leading to whale injuries and deaths and causing economic loss (Baumann-Pickering *et al*. 2015). Detailed classification of echolocation clicks and whistle signalling should make acoustic encounters of these whales identifiable to species level, enabling better long-term monitoring and decreasing bycatch.

3 Aims of the study

The overall aim is to advance the understanding of how middle-ear properties determine sound transfer into the whale inner ear, and how possible left-right asymmetries of the middle ears may improve directional hearing in whales as well as in terrestrial mammals. The specific aims of the three original papers are:

I. *To study mechanical properties of the bony tympano-periotic complex (TPC)*: (1) the amplitude and phase of the vibrational response at different locations to vibrations of different frequencies applied at one end; (2) the relative stiffness of the tympanic and periotic bones. The main question is whether the vibration response of the TPC shows a topographical pattern relevant to the transfer of different sound frequencies into the inner ear.

II. *To study whether important parameters of the Globicephala middle-ear ossicular chain differ between the two ears*, with attention to possible sex and age differences. The parameters measured were the weights and lengths of the ossicles and inter-ossicular angles. The main question is whether there are left-right asymmetries that could support directional hearing.

III. *To put the question of left-right asymmetry serving directional hearing in a broader biological context* by measuring middle-ear parameters of both ears of two mammals: *Ovis aries*, the domestic sheep and *Felis catus*, the domestic cat, and comparing these with two echolocating mammals (the hort-finned pilot whale *Globicephala macrorhynchus*, and Mexican free-tailed bat, *Tadarida brasiliensis mexicana*) The choice and significance of the species selected for this comparative study are explained in the original paper.

4 Materials and methods

For methodological details the Reader is referred to the original papers. Here only a very brief summary is given. Related to papers (I) and (II), I would especially like to point out the statistical power provided by the large number of whale TPC's available to us. This is a rare biological collection, which it took us five years to get permission to obtain legally, with CITES II limitations.

Papers I and II

The studies are based on 32 freshly harvested and fixed pairs of the peripheral hearing organs (tympano-periotic complexes, TPCs) of *Globicephala macrorhynchus*, legally obtained in Taiji Japan.

Paper I describes a laser vibrometry experiment in which we elucidate the topography of vibration frequency responses on the bony TPC of the short-finned pilot whale *Globicephala macrorhynchus.* A piezoelectric-crystal-based vibrator was surgically attached to a location on the cochlea near the exit of the acoustic nerve. The crystal delivered vibrational pulses through continuous sweeps from 5 to 50 kHz. The vibration response was measured as a function of frequency by laser Doppler vibrometry at five points on the TPC and analyzed with respect to both amplitude and phase. In addition, the stiffness of seven TPCs was determined by placing them in a crushing instrument between two metal plates to which a downward force was applied at a predetermined rate.

Paper II reports morphometric features of the malleus, incus and stapes in the pairs of TPC's from each individual. We compared left to right ears and measured the weights and lengths of the

ossicles and the stapes footplate areas. We also calculated the angles between the incudes and stapes, based on computed tomography (CT) images of the ears. Data were analyzed by t-test, sign test and Reduced Major Axis Regression (RMA) analysis.

Paper III

Paper III reports morphometric measures of the ossicles like those obtained in *Globicephala* and *Tadarida* for two species hearing in air and walking on land: *Felis catus* (the domestic cat), and *Ovis aries* (the domestic sheep). The middle ear bones of the latter two species were prepared from fresh animals obtained from abattoirs, or humanely euthanized for reasons not related to these studies. Ear pathology was not reported for any of these individual animals. We also did microdissections on cat and sheep ear regions, and provided anatomical description of them. We then measured the weights and lengths of the ossicles and the stapes footplate area, and compared the left and right ears using t-test.

5 Results

5.1 Physical properties of the tympano-periotic complex (Paper I)

Topography of vibration response

In Paper I we found that the bony TPC responds differently in frequency-dependent manner along its length to incoming sound vibrations. From 12–50 kHz the anterolateral tympanic plate (nearest to the incoming echoes of the animal) responded most sensitively, while amplitudes decreased towards the posterior part of the TPC.

The result that the largest vibration amplitudes are found on the tympanic side lends support to the theory of the four-bone model of Hemilä *et al*. (1999). This may form part of the levers operating in the middle ear: the vibration of the tympanic plate and the lever it forms, according to the four bone model, requires that the upper part of the plate is somewhat flexible in relation to the periotic bone, where there are contacts between these two, or where the tympanic plate has very thin spots.

We propose that the reduction in vibratory displacement between locations D versus B and E (see Fig. 2 A-C and Fig. 5 in Paper I) is part of a “third lever” operating in the whale middle ear. The gain in force is the reciprocal of the displacements at Loc D and Locs B, E. the force exerted on the stapes will be enlarged as $F_1 \times L_1 = F_2 \times L_2$ (F = Force, L = the length of the lever arm). In marine mammals this increase in force would not contribute to impedance matching, but aid in moving the stiffly connected ossicles, which are characteristic of ears using high frequencies for echolocation (Hemilä *et al*. 2001).

Stiffness experiment done on the TPC's. The role of the involucrum.

Our findings suggest that the periotic bone is about five times stiffer that the tympanic bone; in the latter, the involucrum is indeed stiffer than the tympanic plate, and yields to pressure well after and

to a greater load, compared with the tympanic plate. The assumptions of Hemilä *et al*. (1999) are well supported by this part of our experiment.

5.2 The degree of asymmetry between *Globicephala* middle ears and its possible significance for directional hearing (Paper II)

We measured four characters of the auditory ossicles of 32 pairs of the peripheral hearing organ of *Globicephala macrorhyncus*: weight, length, area of the stapes footplate, and the angle between the incus and stapes. The malleus was heavier, and the weight ratio of (malleus + incus) over stapes was greater on the left side. The inter-ossicular angle between the incudes and stapes was also larger on the left side compared to the right. These left-right differences create a dual lever system in the middle ears. We propose how these asymmetries may help this obligatory marine-dwelling animal to locate the source of its prey, especially in the vertical plane.

5.3 Mammals with symmetrical and asymmetrical middle ears (Paper III)

The directional asymmetry found in *Globicephala* ossicles has to be compared with other species in order to ascertain whether it is a "one-off" phenomenon, and if not, if there is any common denominator for species where it exists. The number of mammals potentially available for comparative studies is endless, and we had to limit comparisons to three others: in two we have actually harvested the ossicles and measured their weights and lengths. In a third one we relied on a previous study. The species are: 1) *Ovis aries*, the domestic sheep; 2) *Felis catus*, the domestic cat; 3) *Tadarida brasiliensis*, a South American insectivorous bat. In addition, we briefly relate the results to the asymmetric placement of the external ear meatus on the skull in *Tyto alba*, the barn owl.

The reason for choosing the sheep is that it is an extant artiodactyl close enough to the one which gave rise to the order Cetacea (Thewissen *et al*. 2007). Fresh material was available from local abattoirs. The reason for choosing the domestic cat is that it is a carnivore like the odontocete. We wanted to check whether these features are common to predators, and not limited to the marine habitat. Our measurements of the middle ear ossicles of these two species indicated no asymmetry between the left and right side.

The bat uses ultrasonic sound waves to navigate and hunt like *Globicephala* and other odontocetes do underwater. Lifshytz *et al*. (2000) found directional asymmetry between the left and right ossicles, such that the left is heavier than the right, much the same as we have found in *Globicephala*. This might suggest that it could be a feature of animals using echolocation (biosonar).

Comparison was made with the barn owl, an avian species, which by definition has only the left and right columella to compare, and in this respect does not fit our search. However, it has both its pinnae anatomically placed on the bone skull in a very asymmetric way (Norberg 1978). Thus ear asymmetry in a wider sense is not limited either to mammals, water, or echolocating species. A possible generalization from the species considered here is that ear asymmetries are characteristic of

animals hunting “in three dimensions” in the dark, i.e., relying on hearing for localizing and capturing prey in open water- or air-space (*Globicephala*, *Tadarida* and *Tyto*) under light conditions where vision is unreliable.

6 Discussion

6.1 Functional morphology of the *Globicephala* middle ear

Our results are generally consistent with the four-bone model of Hemilä *et al*. (1999) with some modifications.The model is plausible because it explains the role of several anatomic features and allows realistic fits to the audiograms of several odontocetes (Hemilä *et al*. 2001). However, Cranford *et al*. (2010) raise two major problems with the model. The most important is the assumption that the malleus moves in a simple manner parallel to, and along the axis of the processus gracilis, which is contrary to the complex family of vibrations suggested by the calculations of Cranford *et al*. (2010). The other problem, in their view, is the proposal that sound acts upon the TPC only at one location, the outer lip of the tympanic bulla. Our results modify this picture by showing that, at high (but not lower) frequencies, displacement amplitudes are indeed highest at the anterolateral lip of the tympanic bulla and decrease sequentially through the center of the TP and thence to the cochlea (Figs. 2 and 5 B-C in Paper I).

Obviously, if the whole system were equally rigid, no relative motion would take place. Differential enhancement of vibrations of the thin lateral wall of the tympanic bone is due to the thick medial part called the involucrum. This thick involucrum is an early feature of whale evolution, present already in pakicetids, and actually even in the ancestors of whales. The even higher rigidity of the periotic bone goes well together with this reasoning, contributing to the movement of the most flexible part of the complex (forming the first lever in the system, the second lever being the ossicular chain according to the Hemilä model).

Our results show that the response pattern of the TPC to sound energy indeed varies strongly with the frequency of the sound. The frequency range where the response amplitudes of the TPC decrease in an orderly fashion from the anterolateral edge towards the cochlea (> 30 kHz, see Fig. 5 in Paper I) encompasses both the optimal hearing range and the main range of vocalizations of *Globicephala*. Audiograms of healthy animals show maximal hearing sensitivity around 40 kHz, even higher for juveniles (see 2.4.4 above: Pacini *et al*. 2010; Schlundt *et al*. 2011; Greenhow *et al*. 2014). Important components of vocalizations lie at even higher frequencies (see 2.2.3 above).

6.2 The left-right asymmetry of *Globicephala* middle ears in a general classification of asymmetries

Paper II demonstrates asymmetry between the middle ears of *Globicephala*, and considers if and how this might enhance the exceptional ability of this animal to localize the origin of external incoming sounds as well as the echoes from its own vocalizations.

Phenomena of asymmetry in animals which possess bilateral symmetry have been reviewed and classified by Van Valen (1962), Neville (1976) and Efimov *et al*. (1987). In *directional asymmetry* (DA) there is a systematic difference between the right and left sides (e.g., the mammalian heart); in *antisymmetry* (AS) the right and left sides differ but their roles are reversible (e.g., right- or left-handedness in humans); in *fluctuating asymmetry* (FA), which is ubiquitous, the population average for the right and left sides are equal (i.e. the signed difference averages zero). Some conspicuous cases of DA and AS are obviously functional, such as the differently sized claws of *Uca* crabs (Davis 1987), or the grossly asymmetrical ears of the owls, shown in *Tyto* to support excellent sound localization in the vertical direction (Norberg 1977; Knudsen and Konishi 1979; Konishi 1993). Fig. 4 illustrates the outer-ear asymmetry of another owl species, the oriental bay owl *Phodilus badius*, where the different vertical localization and shape of the ear openings in the skin have been made visible by removal of the feathers

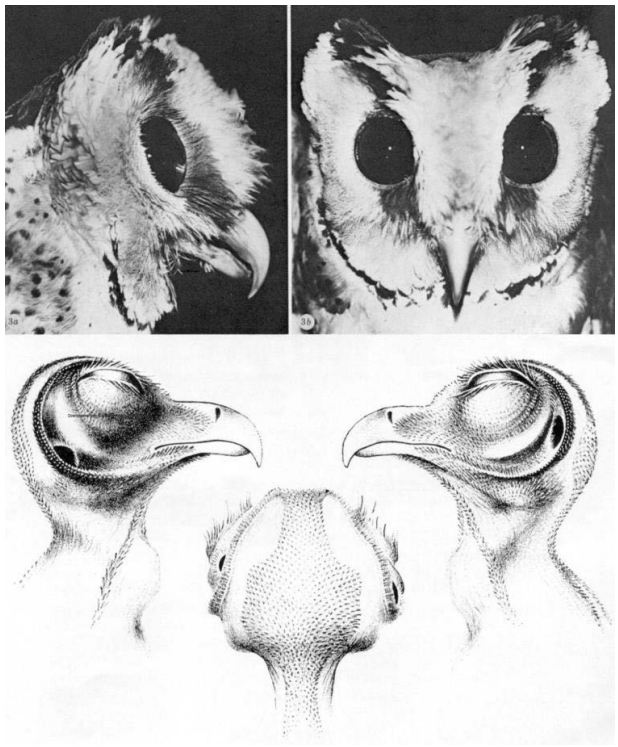


FIGURE 3*a* and *b*. *Phodilus badius*. The form of its facial disk and ruff is unique among owls. The eyes are enormously large, and in this respect differ strikingly from the relatively small eyes in *Tyto alba* (figure 5). Captive owl, San Diego Zoo, July 1974. Photo: R. Å. Norberg.

FIGURE 4. Lateral and posterior views of the head of *Phodilus badius*. The feathers have been removed to show the different vertical locations of the ear openings in the skin. Below the left ear opening in the skin, and above the right one, there is a depression that is located immediately behind the eye and the postorbital process. Behind the ear openings in the skin there is a curved, dermal ridge on which the facial ruff feathers attach. Figures reproduced with minor changes from Pycraft (1903*b*, Plate 2).

Figure 4. Asymmetry relating to the place of the bony base of the pinnae on the skull, as an "alternative" option to deviate from symmetry and enjoy its benefits... From Norberg (1977), Top photographs by Norberg, bottom drawings by Pycraft (1903), reproduced after Norberg (1977).

However, as with many other adaptations in animals, the question arises whether the evolutionary stages are also functional (Mayr 1960) and indeed, whether they exist. Most studies of asymmetry have focussed on FA, viewed as a measure of weakened homeostasis and developmental control; DA has been viewed as a secondary, or as a complication to be neutralized (Palmer and Strobeck 1986). No functional role was proposed in these studies; on the contrary, it has been suggested that, to the extent that DA is heritable, it is selected against, particularly where it interferes with functions that depend on the symmetry of the body, such as most locomotion (Jolicoeur 1963; Leamy 1984). However, in a thorough study of natural selection on morphological phenotype of a lizard, Fox (1975) demonstrated that the frequency of asymmetry was not changed by differential survival of the asymmetric individuals. Whereas FA is generally regarded as expressing a weakness of the genetic control of ontogeny, statistically valid DA, unless phenotypic owing to external effects (Fox *et al*. 1961), may be interpreted as genetically determined. In a study of DA in reptiles (Werner *et al*. 1991) DA was found in five or six character-taxon combinations (out of twelve examined) in three congeneric species of gekkonid lizards in Israel and Sinai. The DA differed in sign between parapatric taxa, and therefore can hardly be explained as phenotypic. This supposedly genotypic DA presumably is either adaptive, or non-adaptive and evolutionarily neutral. As Simpson (1953) has pointed out, non-adaptive characters are expected to constitute a minority and to distinguish mainly low-level taxa.

In our study, we found DA in four out of six mensural characters of the middle ear of *Globicephala* and when considering also compound characters, the total was six out of eight. Asymmetry of the head has been established in Archaeoceti (Fahlke *et al*. 2011) and we can assume that after 34-35 million years of existence in modern odontocetes, these characters have a functional role in hearing underwater. This is likely especially because asymmetry of the external (in land vertebrates) and middle ears may attain deleterious levels, as optimal function of the hearing organ depends on dimensions (Wever and Werner 1970; Werner and Wever 1972; Werner *et al*. 2008). Our modelling suggests that the middle-ear asymmetries might support disambiguation of sound direction in *Globicephala*.

6.3 Forces that shape middle-ear morphology

Surgically oriented, evidence-based human research

As explained above (5.3), a possible generalization based on the study of four mammals in Paper III plus previous results on the barn owl (Knudsen and Konishi 1979), is that ear asymmetries are useful for animals hunting in "three dimensions" in the dark, i.e., using hearing for localizing and capturing prey in open water- or air-space (*Globicephala*, *Tadarida* and *Tyto*) under low-light conditions where vision is unreliable. Importantly, left-right asymmetries in the weights of the ossicles were not found in cats and sheep. Even if studies of further species might disrupt the present generalization, the fact is that asymmetry is not a universal phenomenon, and where it is present, it should be explained as far as possible.

However, interpretation of asymmetries is difficult for many reasons. Here I wish to expand the comparative and general discussion beyond what space would permit in the original paper. A good starting point is the best-studied of all species, *Homo sapiens*. Human surgically-oriented studies have general insights to offer, and conversely the understanding of human auditory function in health and disease may benefit from comparative knowledge from other species. The *asymmetrical structure of the human stapes* (see Fig. 1 A) was one of the questions that motivated Hüttenbrink (1996) in a microanatomical analysis of the ossicles of 175 species (mostly mammals and birds, collected by C. F. Werner in the first half of last century). His main idea is that the anatomical structure of the middle ear is not only influenced by its function as sound transmitter, but also shaped by non-acoustic forces and static loads depending on the environment. For example, ossicles of aquatic mammals are more solid and denser than in land mammals. Drawing a parallel to the relation of skeletal bone structure to static load and muscle force, he explains the asymmetry of the human stapedial arch as an adaptation to the pull of the stapedius muscle.

There are other intriguing observations from humans. In a study of ossicular chain articulations from the tympanic membrane to the oval window (23 subjects), Ramirez and Ballesteros (2010) found significant lateral asymmetry in (only) one measure, the articular area of the incudo-malleal joint (larger on the left side). They provide no functional interpretation and the observation might be unimportant, but in view of the complexity of middle-ear ossicle movements in some regimes, it is clear that such parameters could affect hearing in certain frequency ranges. Cai *et al*. (2010) simulated the motion modes of the human middle-ear structures with a finite element (FE) model based on 3D reconstruction obtained from micro CT. At high frequencies they found complexities suggesting that middle-ear motion modes and dynamics are determined by specific anatomical features (saddle shape of the malleo-incudal joint, asymmetry of the eardrum) in conjunction with the three-dimensional inertial properties of the ossicles. They propose that the twisting mode of the malleus and incus serves an inertia-reducing function in humans and other larger mammals. Puria and Steele (2010) similarly modelled the motion modes of several mammals, showing that in cat and human the ossicular moment of inertia calculated for the unfused malleus are 5–6 times smaller for rotations about an inferior–superior axis than for rotations about the other two orthogonal axes. They suggest that the high-frequency hearing limit of a given mammalian species can in part be understood in terms of morphological co-adaptations of the eardrum and ossicular chain. Obviously, these appear as potentially important effects on top of the basic dependence of the high-frequency limit on ossicular mass expressed by the Hemilä *et al*. (1995) relation (relation (5) under 2.3 above). This relation has been shown to explain a large percentage of the variance for 26 land mammals, but indeed the cat is an outlier with a higher high-frequency hearing limit than predicted.

The studies of Cai *et al*. (2010) and Puria and Steele (2010), including the method (micro CT and FE modelling), are similar to the work of Cranford et al. (2010) on *Tursiops*. There too, different parts of both the TPC and the middle ear ossicles come into intricate play at higher frequencies. Any deviation from symmetry must surely have an effect on transmission, as calculated in Paper II based on the four-bone model of Hemilä *et al*. (1999), be it in the eardrum (not measured by myself), the 3D inertial properties of the ossicles, or the effect the ossicle mass has upon the dynamics of the middle-ear response, all of which play a crucial part in shaping the auditory signals travelling to the brain. The significance of differing ossicular masses on the final shape of the sound signals from the two ears and its interpretation in the auditory cortex cannot be overemphasized.

The symmetry of sheep and cat middle ears

The domestic sheep. We found perfect left-right symmetry in the ossicles of *Ovis aries*. The sheep is an extant artiodactyl that in this context closely enough resembles the ancestral Eocene whales, suggesting that the asymmetry persisting in modern odontocetes is related to life in water. Further, there is an applied aspect worth mentioning about the sheep: Péus *et al*. (2017) have shown that it can be an appropriate, large animal model for research and development of implantable hearing devices in people. Our finding contributes to validating this model, since perfect symmetry means that testing a prosthesis (e.g. stapes) on one side of a live sheep can be regarded as sufficient proof that it can be safely used on the contralateral side. This needs to be emphasized, because as shown in this thesis, assuming symmetry without actual measurements can lead to erroneous results (see also Werner *et al*. 2001).

The domestic cat. The left-right symmetry we found in *Felis catus* shows that being a carnivore and active (also) in dim light is not necessarily correlated with asymmetric middle ears. A review of middle-ear performance in cats covering a size range from 3 kg (sand cat) to 180 kg (tiger) lends support to the idea that these land carnivores do not need middle-ear asymmetry to locate the source of incoming sound (Huang, PhD thesis, M.I.T. 1999). For example, the very mobile ear pinnae of the domestic cat allow using the head-related transfer function (HRTF) in a manner not available to odontocetes.

A final comment

Finding asymmetries of any sort does require our attention, because any deviation from perfect symmetry in bilateral organs implies a deviation from the basic scheme, and in this respect warrants an explanation. In the present study we consider only four mammalian species, but propose to open this topic for further research. First we must carefully describe what we see, then seek an explanation as to its existence, viewed not in a narrow perspective, but rather in the light of the animal's entire *Umwelt* (*sensu* Alexander von Humboldt and Jakob Johann von Uexküll). The comparative question, where else this biological phenomenon (ear asymmetry) can be found and where not, challenges us to provide a comprehensive theory for the role of asymmetry in improving the efficiency of an animal's auditory system in the full context of its natural history. More generally, one has to consider which communication modality (visual, olfactory, auditory, tactile, electrical) is best suited for the animal in its particular environment.

7 Conclusions

I. The topography of vibration responses on the tympano-periotic complex (TPC) of *Globicephala macrorhynchus* was frequency-dependent. In a high-frequency range (> 30 kHz), which encompasses the best hearing range as well as the predominant vocalization range of the animal, there was a regular pattern such that the anterolateral lip of the tympanic plate (TP) responded with the largest amplitudes, while amplitudes decreased in orderly manner more posteriorly across the TP with a minimum near the cochlea. This is consistent with a lever mechanism trading motion amplitude for force at the attachment to the ossicular chain, contributing to the sensitivity of the animal to high frequencies. At lower frequencies (< 12 kHz) the pattern of vibration amplitudes was less regular and basically inverted. The motion patterns are determined by the relation of the thin TP to the stiff medial part of the tympanic bone (the involucrum) and the even stiffer periotic bone.

II. Significant left-right asymmetries were found for *Globicephala macrorhynchus* in the weights of the malleus and incus (heavier in the left ear), the ratio of (malleus + incus) weight over stapes weight (greater in the left ear), and the angle between the incus and stapes. Modelling shows that these asymmetries might support disambiguation of sound direction especially in the vertical plane.

III. Comparisons between left and right middle ears were found to show symmetry both for *Felis catus* and *Ovis aries*, when weights of the malleus and incus, and the ratio of (malleus + incus) weight over stapes weight were studied. This was taken to suggest that hunting in the dark, using mainly auditory cues, does not necessarily assume left-right asymmetry in the ear structures. Neither is asymmetry expressed in an artiodactyl that can be regarded to be a close relative of cetaceans. Dolphins and insectivorous bats have a crucial advantage of left-right asymmetry between the ears, as they rely more exclusively on auditory cues than the cat and sheep, whose sensory spaces are based on a wider combination of sensory cues received through different sensory modalities.

8 References

Aguilar Soto, N., Johnson, M.P., Madsen, P.T., Diaz, F., Dominguez I., Brito, A., Tyack, P. (2008). Cheetahs of the deep sea: deep foraging sprints in short-finned pilot whales off Tenerife (Canary Islands). J. Anim. Ecol. 77, 936–947. doi: 10.1111/j.1365-2656.2008.01393.x.

Aroyan, J.L. (2001). Three-dimensional modeling of hearing in Delphinus delphis. J. Acoust. Soc. Am. 110:3305-3318.

Au, W. W. L., Branstetter, B. K., Benoit-Bird, K. J., and Kastelein, R. A. (2009). Acoustic basis for fish prey discrimination by echolocating dolphins and porpoises. J. Acoust. Soc. Am. 126:460–467.

Au W.W., Branstetter, B., Moore, P.W., Finneran, J.J. (2012) The biosonar field around an Atlantic bottlenose dolphin (Tursiops truncatus). J. Acoust. Soc. Am. 131:569–576.

Au, W. W. L., Moore, P. W. B. (1984). Receiving beam patterns and directivity indices of the Atlantic bottlenosed dolphin (Tursiops truncatus). J. Acoust. Soc. Am. 75:255–262.

Autrum, H.-J. (1940). Über Lautäusserungen und Schallwahrnehmung bei Arthropoden. II. Das Richtungshören von Locusta und Versuch einer Hörtheorie für Tympanalorgane vom Locustidentyp. Z. vergl. Physiol. 28, 326-352.

Barany, E. (1938). A contribution to the physiology of bone conduction. Acta Otolaryngol. Stockholm Suppl. 38.

Baumann-Pickering, S., Simonis, A.E., Oleson, E.M., Baird, R.W., Roch, M.A., Wiggins, S.M. (2015). False killer whale and short-finned pilot whale acoustic identification. Endang. Species Res. 28:97-108. https://doi.org/10.3354/esr00685

Boran, J., Heimlich, S. (2019). Pilot whales: Delphinid matriarchies in deep seas. In: B. Würsig (ed.) Ethology and Behavioral Ecology of Odontocetes. Springer Nature Switzerland, Cham, pp. 281-304.

Bradbury, J.W., Vehrenkamp, S.L. (2011). Principles of animal communication (2nd ed.). Sinauer Associates.

Branstetter, B.K., Mercado, E. (2006). Sound localization by cetaceans. Int. J. Comp. Psychol. 19:26-61.

Brill, R. L., Sevenich, M. L., Sullivan, T. J., Sustman, J. D., and Witt, R. E.(1988). Behavioral evidence of hearing through the lower jaw by an echolocating dolphin (Tursiops truncatus). Marine Mammal Sci. 4:223–230.

Brill, R. L., Moore, P. W. B., Helweg, D. A., and Dankiewicz, L. A. (2000). Mapping acoustic sensitivity about the dolphin's head: A look at the peripheral hearing system. J. Acoust. Soc. Am. 107, 2786.

Bullock TH, Grinnell AD, Ikezono E, Kameda K, Katsuki Y, Nomoto M, Sato O, Suga N, Yanagisawa K. (1968). Electrophysiological studies of central auditory mechanisms in cetaceans. Z. Vergl. Physiol. 59:117–156.

Butler, R. A. (1999). The unfolding of an auditory illusion. Perspectives in Biology and Medicine 42:157-173.

Cai, H., Jackson, R.P., Steele, C., Puria, S. (2010). Proc. COMSOL Conference 2010, Boston.

Carr, C.E., Konishi, M. (1990). A Circuit for Detection of Interaural Time Differences in the Brain Stem of the Barn Owl. J. Neurosci. 70:3227-3246.

Christensen-Dalsgaard, J.C., Manley, G.A. (2005). Directionality of the lizard ear. J, Exp, Biol. 208:1209-1217.

Coombs, E.J., Clavel, J., Park, T., Churchill, M. Goswami, A. (2020). Wonky whales: the evolution of cranial asymmetry in cetaceans. BMC Biol. 18:86. https://doi.org/10.1186/s12915-020-00805-4.

Cranford, T.W., Amundin, M,, Norris, K,.S. (1996). Functional morphology and homology in the odontocete nasal complex: Implications for sound generation. J. Morphol. 228:223–285

Cranford, T.W., McKenna, M.F., Soldevilla, M.S., Wiggins, S.M., Goldbogen, J.A., Shadwick, R.E., Krysl, P., St. Leger, J.A., Hildebrand, J.A. (2008). Anatomic geometry of sound transmission and reception in Cuvier's beaked whale (Ziphius cavirostris). Anat. Rec. 291:353-378.

Cranford, T.W., Krysl, P., Amundin, M., (2010). A new acoustic portal into the odontocete ear and vibrational analysis of the tympanoperiotic complex. PLoS One 5 (8) e 11927:1-26.

D'Amico, A.D., Gisiner, R., Ketten, D.R., Hammock, J.A., Johnson, C., Tyack, P., Mead, J. (2009). Beaked whale strandings and naval exercises. Aquat. Mamm. 35:452–472.

Davis, T.A. (1987). Laterality in Crustacea. Proc. Indian natn. Sci. Acad. (B) 53:47-60.

Dong, J., Song, Z., Li, S., Gong, Z., Li, K., Zhang, P., Zhang, Y., Zhang, M. (2017). Acoustic properties of a short-finned pilot whale head with insight into temperature influence on tissues' sound velocity. J. Acoust. Soc. Am. 142:1901-1912. doi: 10.1121/1.5005509.

Efimov, V.M., Akimov, I.A., Galaktionov, Yu.K. (1987). [Formal hierarchical classification of the types of bilateral asymmetry in biological objects.] Dokl. Akad. Nauk. Ukr. SSR, Ser. B, Geol. Khim. Biol. Nauki 1987 (3): 64-66. [In Russian.]

Fahlke, J.M., Gingerich, P.D., Welsh, R.C., Wood, A.R. (2011). Cranial asymmetry in Eocene archaeocete whales and the evolution of directional hearing in water. Proc. Natl Acad. Sci. U S A. 108:14545–14548.

Foskolos, I., Aguilar de Soto, N., Madsen, P.T., Johnson, M. (2019). Deep-diving pilot whales make cheap, but powerful, echolocation clicks with 50 μL of air. Sci. Rep. 9:15720 | https://doi.org/10.1038/s41598-019-51619-6.

Fox, S.F. (1975). Natural selection on morphological phenotypes of the lizard Uta stansburiana. Evolution 29:95-107.

Fox, W.. Gordon, C., Fox, M.H. (1961). Morphological effects of low temperatures during the embryonic development of the garter snake, Thamnophis elegans. Zoologica, N.Y. 46:57-71.

Geisler, C.D. (1998). From sound to synapse: physiology of the mammalian ear. New York: Oxford University Press

Gelfand, S. A. (2004). Hearing: An Introduction to Psychological and Physiological Acoustics. 4th Edition New York: Marcel Dekker.

Giraud-Sauveur, D. (1969). Recherches biophysiques sur les osselets des Cétacés. Mammalia 33:285–340.

Greenhow, D.R., Brodsky, M.C., Lingenfelser, R.G., Mann, D.A. (2014). Hearing threshold measurements of five stranded short-finned pilot whales (Globicephala macrorhynchus). J. Acoust. Soc. Am. 135:531-536.

Grothe, B., Pecka, M., McAlpine, D. (2010). Mechanisms of sound localization in mammals. Physiol Rev 90:983–1012. doi:10.1152/physrev.00026.2009.

Heffner, H.E., Heffner, R.S. (2016).. The Evolution of Mammalian Sound Localization. Acoustics Today 12:20-27.

Heffner, R. S., Masterton, R.B. (1990). Sound localization in mammals: Brain-stem mechanisms. In M. A.Berkley and W. C.Stebbins (eds): Comparative perception, Vol. 1. Basic mechanisms. John Wiley & Sons.

Hemilä, S., Nummela, S., Reuter, T. (1995). What middle ear parameters tell about impedance matching and high-frequency hearing. Hear. Res. 85:31–44.

Hemilä, S., Nummela, S., Reuter, T. (1999). A model of the odontocete middle ear. Hear. Res. 133:82-97.

Hemilä, S., Nummela, S., Reuter, T., (2001). Modelling whale audiograms: effects of bone mass on high-frequency hearing. Hear. Res. 151:221-226.

Hemilä, S., Nummela, S., Reuter, T. (2010). Anatomy and physics of the exceptional sensitivity of dolphin hearing (Odontoceti: Cetacea). J. Comp. Physiol. A 196, 165-179.

Heyning, J.E. (1989). Comparative facial anatomy of beaked whales (Ziphiidae) and a systematic revision among the families of extant Odontoceti. Natural History Museum of Los Angeles County Contributions in Science 405:1-64.

Houser, D.S.. Finneran, J., Carder, D. ,Van Bonn, W., Smith, C., Hoh, C., Mattrey, R., Ridgway, S. (2004). Structural and functional imaging of bottlenose dolphin (Tursiops truncatus) cranial anatomy. J. Exp. Biol, 207:3657–3665.

Huang, G.T. (1999). Measurement of middle-ear acoustic function in intact ears: application to size variations in the cat family. PhD thesis, Massachusetts Institute of Technology.

Hüttenbrink, K.B. (1996). Ein Vergleich der Morphologie von Säugetier- und Vogel-Gehörknöchelchen und Überlegungen zur asymmetrischen Form des menschlichen Steigbügels. Laryngorhinootologie 75:123-8.

Jensen, F. H., Bejder, L., Wahlberg, M., Aguilar Soto, N., Johnson, M., Madsen, P. T. (2009). Vessel noise effects on delphinid communication. Mar. Ecol. Progr. Ser. 395:161–175.

Jensen, F. H., Marrero, J.M., Aguilar Soto, N., Johnson, M., Madsen, P. T. (2011). Calling under pressure. Proc. R. Soc. B 278:3017-3025.

Jolicoeur, P. (1963). Bilateral symmetry and asymmetry in limb bones of Martes americana and Man. Rev. Can. Biol. 22:409-432.

Ketten, D.R., 1997. Structure and function in whale ears. Bioacoustics 8:103-135.

Ketten, D.R., 2000. Cetacean ears. In: Au, W., Popper, A., Fay, R. (Eds.), Hearing by Whales and Dolphins. Springer, New York, NY, pp. 43-108

Kinsler, L.E.; Frey, A.R.; Coppens, A.B., Sanders, J.V. (2000). Fundamentals of Acoustics, 4th Edition. New York: John Wiley & Sons. pp. 120–121. ISBN 0-471-84789-5.

Knudsen, E.I., Blasdel, G.G., Konishi, M. (1979). Sound localization by the barn owl *Tyto alba* measured with the search coil technique. J. Comp. Physiol. A 133, 1-11.

Knudsen, E.I., Konishi, M. (1979). Mechanisms of sound localization in the barn owl (*Tyto alba*) J. Comp. Physiol. A 133:13-21.

Konishi, M. (1993). Listening with two ears. Scientific American 268:34-41.

Krings, M., Rosskamp, L. and Wagner, H. (2018). Development of ear asymmetry in the American barn owl (*Tyto furcata pratincola*). Zoology 126: 82-88.

Leamy, L. (1984). Morphometric studies in inbred and hybrid house mice. V. Directional and fluctuating asymmetry. Am. Nat. 123:579-593.

Lees, S., Ahern, J.M., Leonard, M. (1983). Parameters influencing the sonic velocity in compact calcified tissues of various species. J. Acoust. Soc. Am. 74:28–33.

Lewis, B. and Coles, R. (1980). Sound localization in birds. Trends Neurosci. 3, 102-105.

Lifschytz, T., Tchernov, E., Werner, Y.L. (2000). Directional asymmetry of middle-ear ossicles in the free-tailed Mexican bat Tadarida brasiliensis. Israel Journal of Zoology 46: 166-167 (English abstract of MSc thesis written in Hebrew).

MacLeod, C.D., Reidenberg, J.S., Weller, M,, Santos, M.B, Herman, J., Goold, J., Pierce, G.J. (2007). Breaking symmetry: The marine environment, prey size, and the evolution of asymmetry in cetacean skulls. Anat. Rec. (Hoboken) 290:539-545.

Madsen, P.T., Carder, D.A., Bedholm, K., Ridgway, S.H. (2005). Porpoise clicks from a sperm whale nose – convergent evolution of 130 kHz pulses in toothed whale sonars? Bioacoustics 15:195–206.

Masterton, I.B., Heffner, H., Ravizza, R. (1969). The evolution of human hearing. J. Aeoust. Soc. Am. 45:966—985.

Mayr, E. (1960). The emergence of evolutionary novelties. In: S. Tax (ed..Evolution After Darwin, Vol. I. Chicago: University of Chicago Press. pp. 349–380.

McCormick, J.G., Wever, E.G., Palin, G., Ridgway, S.H. (1970). Sound conduction in the dolphin ear. J. Acoust. Soc. Am. 48:1418–1428.

Mead, J.G. (1975). Anatomy of the external nasal passages and facial complex in the Delphinidae (Mammalia: Cetacea). Smithson. Contrib. Zool. 207:1–72.

Morisaka, T., Connor, R.C. (2007). Predation by killer whales (Orcinus orca) and the evolution of whistle loss and narrow-band high frequency clicks in odontocetes. J. Evol. Biol. 20:1439–1458.

Mead, J.G. (1975). Anatomy of the external nasal passages and facial complex in the Delphinidae (Mammalia: Cetacea). Smithson Contrib Zool 207:1-72.

Møhl B, Au WWL, Pawloski J, Nachtigall PE. (1999). Dolphin hearing: relative sensitivity as a function of point of application of a contact sound source in the jaw and head region. J Acoust Soc Am 105:3421–3424.

Mooney, T. A, Yamato, M., Branstetter, B. (2012). Hearing in cetaceans: from natural history to experimental biology. Advances in Marine Biology, 63:197-246.

Ness, A.R. (1967). A measure of asymmetry of the skulls of odontocete whales. J. Zool., 153:209–21.

Neville, A.C. (1976). Animal symmetry. (Institute of Biology Studies in Biology No. 67) London: Edward Arnold.

New, L.F., Moretti, D.J., Hooker, S.K., Costa, D.P., Simmons, S.E. (2013). Using energetic models to investigate the survival and reproduction of beaked whales (family Ziphiidae). PLoS One 8 (7):e68725

Norberg, R. A. (1977). Occurrence and independent evolution of bilateral ear asymmetry in owls and implications on owl taxonomy. Phil. Trans. Roy. Soc. Lond. Ser. B. 280:375408.

Norberg, R. (1978). Skull asymmetry, ear structure and function, and auditory localization in Tengmalms owl, Aegolius funereus (Linne). Philosophical Transactions of the Royal Society of London: B Biological Sciences, 282, 325-410.

Norris, K.S. (1968). The evolution of acoustic mechanisms in odontocete cetaceans. In: Drake, E.T. (Ed.), Evolution and Environment. Yale University Press, New Haven, CT, pp. 297-324.

Nummela, S. (1995). Scaling of the mammalian middle ear. Hearing Research 85:18-30.

Nummela, S. (1999). Scaling and Modeling of the Mammalian Middle Ear. Ph.D. Dissertation. University of Helsinki.

Nummela, S., Kosove, J.E., Lancaster, T.E., Thewissen, J.G.M. (2004b). Lateral mandibular wall thickness in Tursiops truncatus: variation due to sex and age. Mar. Mammal Sci. 20:491–497.

Nummela, S., Reuter, T., Hemilä, S., Holmberg, P., Paukku, P. (1999a). The anatomy of the killer whale middle ear (Orcinus orca). Hear. Res. 133:61–70.

Nummela, S., Thewissen, J.G.M., Bajpai, S., Hussain, S.T., Kumar, K. (2004a). Eocene evolution of whale hearing. Nature 430:776–778.

Nummela, S., Thewissen, J.G.M., Bajpai, S., Hussain, T., Kumar, K. (2007). Sound Transmission in Archaic and Modern Whales: Anatomical Adaptations for Underwater Hearing. Anatomical Record 290:716–733.

Nummela, S., Wägar, T., Hemilä, S., Reuter, T. (1999b). Scaling of the odontocete middle ear. Hear. Res. 133:71-81.

Pacini, A. F., Nachtigall, P. E., Kloepper, L. N., Linnenschmidt, M., Sogorb, A., Matias, S. (2010). Audiogram of a formerly stranded long-finned pilot whale (Globicephala melas) measured using auditory evoked potentials. J. Exp. Biol. 213:3138-3143.

Palmer, A.R., Strobeck, C. (1986). Fluctuating asymmetry: measurements, analysis, patterns. Ann. Rev. Ecol. Syst. 17:391-421.

Payne, R.S. (1971). Acoustic location of prey by barn owls (Tyto alba). J. Exp. Biol., 54:535-573.

Péus, D., Dobrev, I., Prochazka, L., Thoele, K., Dalbert, A., Boss, A., Newcomb, N., Probst, R., Röösli, C., Sim, J.H., Huber, A., Pfiffner, F. (2017). Sheep as a large animal ear model: middle-ear ossicular velocities and intracochlear sound pressure. Hear. Res. 351:88–97

Popov, V.V., Supin, A.Ya. (1990). Localization of the acoustic window at the dolphin's head. In: Thomas JA, Kastelein RA, editors. Sensory abilities of cetaceans: laboratory and field evidence. New York: Plenum Press. pp. 417–426.

Popov, V. V., Supin, A. Ya., Pletenko, M. G. and Tarakanov, M. B. (2010). Audiogram variability in normal bottlenose dolphins (*Tursiops truncatus*). Aquat. Mamm. 33:24-33.

Puria, S. and Steele, C. (2010). Tympanic-membrane and malleus–incus-complex co-adaptations for high-frequency hearing in mammals. Hearing Research 263:183–190.

Pycraft, W.P. (1903). On the pterylography of *Photodilus*. Ibis (1903), 36-48.

Ramirez, L.M., Ballesteros, L.E. (2010). Human oscicular chain articulations: Asymmetric sound transmission. Inernational Journal of Morphology, 28:1059-1068.

Renaud, D.L., Popper, A.N. (1975). Sound localization by the bottlenose porpoise, Tursiops truncatus. J. Exp. Biol. 63:569-585

Reuter, T., Nummela, S., Hemilä, S. (1998). Elephant hearing. J. Acoust. Soc. Am. 104:1122-1123.

Richardson, W.J., Greene, C.R. Jr., Malme, C.I., Thomson, D.H. (1995). Marine mammals and noise. San Diego: Academic Press.

Ridgway, S., Harrison, R. (eds.) (1999). Handbook of Marine Mammals, Vol. 6: Second book of Dolphins and porpoises. Academic Press, pp. 245-262.

Schlundt, C.E., Dear, R.L., Houser, D.S., Bowles, A.E., Reidarson, T., Finneran, J.J. (2011). Auditory evoked potentials in two short-finned pilot whales (Globicephala macrorhynchus). J. Acoust. Soc. Am. 129:1111-1116. https://doi.org/10.1121/1.3531875.

Simpson, G. G. (1953). The majorfeatures qfedution. New York: Columbia University Press

Supin, A.Y., Popov, V.V. (1993). Direction-dependent spectral sensitivity and interaural spectral difference in a dolphin: Evoked potential study. J. Acoust. Soc. Am. 93:3490–3495.

Supin, A.Ya., Popov, V.V., Mass, A.M. (2001). The sensory physiology of aquatic mammals. Boston: Kluwer Academic Publishers.

Taylor, K. (2013). Directional hearing and head related transfer function (HRTF) in a bottlenose dolphin (Tursiops truncatus). PhD dissertation, University of Hawaii.

Thewissen, J.G.M., Cooper, L.N., Clementz, M.T., Baipai, S., Tiwan, B.N. (2007). Whales originated from aquatic artiodactyls in the Eocene epoch of India. Nature 450:1190-1195. doi:10.1038/nature0634.

Tonndorf, J. (1968). A new concept of bone conduction. Arch. Otolaryngol. 87:49–54.

Tougaard, J., Carstensen, J., Teilmann, J., Skov, H., Rasmussen, P. (2009). Pile driving zone of responsiveness extends beyond 20 km for harbour porpoises (*Phocoena phocoena*, (L.)). J. Acoust. Soc. Am. 126, 11–14.

Tougaard, J., Wright, A.J., Madsen, P.T. (2014). Cetacean noise criteria revisited in the light of proposed exposure limits for harbour porpoises. Mar Pollut Bull. 90: 196-208. https://doi.org/10.1016/j.marpolbul.2014.10.051.

Tyack, P.L. (2009). Effects of human-generated sound on marine mammals. Phys Today 62:39–44.

Tyack, P. (2019). Communication by sound and by visual, tactile, and chemical sensing. In: B. Würsig (ed.), Ethology and Behavioral Ecology of Odontocetes, Ethology and Behavioral Ecology of Marine Mammals. Springer Nature Switzerland, Cham, pp. 25-50. https://doi.org/10.1007/978-3-030-16663-2_2

Van Valen, L. (1962). A study of fluctuating asymmetry, Evolution 16:125-142.

Varanasi, U., Malins, D.C. (1971). Unique lipids of the porpoise Tursiops gilli: differences in triacylglycerols and wax esters of acoustic (mandibular canal and melon) and blubber tissues. Biochim Biophys Acta (Lipids) 231:415–418.

Varanasi, U., Malins, D.C. (1972). Triacylglycerols characteristic of porpoise acoustic tissues: molecular structures of diisovaleroylglycerolides. Science 176:926–928.

Vater, M., Kössl, M. (2004). The ears of whales and bats. In: Echolocation in Bats and Dolphins (J. A. Thomas, C. F. Moss, M. Vater, eds), pp. 89-99. The University of Chicago Press, Chicago and London.

Wagner, G.P., Schwenk, K. (2000). Evolutionarily stable configurations: functional integration and the evolution of phenotypic stability. Evol Biol 31:155–217.

Wartzok, D., Altmann, J., Au, W., Ralls ,K., Starfield, A., Tyack, P.L. (2005). Marine mammal populations and ocean noise: determining when noise causes biologically significant effects. National Academy Press, Washington, DC.

Werner, Y.L. (2001). Minor directional asymmetry in reptiles: a minor personal review. Abstracts, 4th World Congress of Herpetology, Sri Lanka, 2001, pp. 129-130.

Werner, Y.L., Montgomery, L.G., Seifan, M., Saunders, J.C. (2008): Effects of age and size in the ears of gekkotan lizards: Auditory sensitivity, its determinants, and new insights into tetrapod middle-ear function. Pflügers Archiv - European Journal of Physiology, 456:951–967.

Werner ,Y.L. Rothenstein, D. Sivan, N. (1991). Directional asymmetry in reptiles (Sauria: Gekkonidae: Ptyodactylus) and its possible evolutionary role, with implications for biometrical methodology. J. Zool. Lond. 225:647-658.

Werner, Y.L., Wever, E.G. (1972). The function of the middle ear in lizards: Gekko gecko and Eublepharis macularius (Gekkonoidea). J. Exp. Zool. 179:1-16.

Wever. E. G. & Werner, Y. L. (1970). The function of the middle ear in lizards: Crotaphytus collaris (Iguanidae). J. Exp. Zool. 175: 327-342.

Wisniewska, D. M., Johnson, M. P., Nachtigall, P. E. & Madsen, P. T. (2014). Buzzing during biosonar-based interception of prey in the delphinids *Tursiops truncatus* and *Pseudorca crassidens*. J. Exp. Biol. 217, 4279–4282.

9 Postscript and Acknowledgements

Luke 8:8

"Still other seed fell on good soil. It came up and yielded a crop, a hundred times more than was sown." When he said this, he called out, "Whoever has ears to hear, let them hear."

Mark 7:16

If any man has ears to hear, let him be listening and let him perceive and comprehend by hearing.

It is quite amazing to find that our ancient prophets have made the clear distinction between the passive reception of changes in air pressure, which enable us to HEAR, and the conscious, active effort to actually LISTEN and COMPREHEND what we hear. This thesis is a very modest effort to try to bridge this gap in the life history of Odontocetes, the toothed whales, namely translate hearing to listening, thereby enhancing their inclusive fitness.

My understanding of the title "Doctor of Philosophy" is that it should not be limited to merely having a theory, collecting data, analyzing it and presenting the conclusions for peer review. Philosophy in the Concise Oxford Dictionary is defined as: "Seeking after wisdom or knowledge, esp. that which deals with the most general causes and principles of things and ideas, and human perception and knowledge of them…" From Greek: *Sophia: Wisdom.* Aristotle, one of the greatest philosophers ever, advocated the seeking of knowledge for knowledge's sake, based on our observations of the world around us. I went into this lifelong adventure for only one reason: The subject aroused an immense curiosity in me, when, back in 1978, during my First degree in Biology and Chemistry in Birkbeck College, University of London, I first came across dolphins, in the Mammals course project called: "Underwater Communication in Marine Mammals". I was hooked on the subject, as on dolphins, ever since. Anyone who touched the silky skin of *Tursiops*, will have experienced the magic which these creatures spread around them. I make no apologies for introducing some of my (philosophical) thoughts in many places throughout the thesis, and see this as an integral part of "Fulfilling the (formal) requirements of the University" to attain this degree.

In this moment of fulfilment, my deepest gratitude goes to my soul mate, Spyros Elia MBE, who kept a life line open for me at all times, during those lean mean years, and without whose loving and caring friendship I would not have survived. Thank you my wise and gentle Hellenic friend, and here it is; I kept my promise to you and Greg from 1978, that one day I shall have something original to say about dolphins… To Dr. R. Aronson, whose serenity and navigational ability, matched only by my beloved whales, have kept my boat on an even keel, bringing it to much safer shores.

I quote from the introduction to the PhD thesis of Professor Kristen Taylor (HRTF in a Bottlenose dolphin, *Tursiops truncatus*, 2013): "It takes a village to raise a child". And what an amazing village mine has been: First and foremost, Yehudah L. Werner, Professor Emeritus of Zoology, Department of Ecology, Evolution and Behavior, The Hebrew University of Jerusalem. Professor

Werner is a world authority on reptilian and amphibian hearing, especially the songs of Geckos. The HUJ has no specialist in Cetaceans as such, less so their hearing. Yehudah has offered to be my "Academic umbrella" for this, our joint effort and journey for the last twenty years, at the end of which he himself became quite an expert on Cetacean songs, too. He recently summed up his experience saying with his typical humor: "Yes, I have found the dolphin a very unusual gecko"… The thesis, Yehudah, bears all the attributes and hallmarks which all of your past and present students, colleagues and readers can recognize as "Made in Yehudah" (comparable to e.g. "Made in Havana" when talking about the best quality cigars…). Your relentless demand for perfection, both in the shape and contents of written material, has enabled the thesis to reach its present level. I am indebted to your overwhelming patience during the long years you have looked over the process of creating this work, your endless knowledge and your fatherly, stern attitude and advice, using the stick wherever needed, so that the carrot at the end will have been deservedly earned. It would not have been possible without you.

I am grateful to all the people, friends and family and of course the scientists who helped along this personal journey of mine: To Mr. Tadashi Araki, Tokyo, who enabled the acquisition of the biological material, which it took five years to legally obtain, in accordance with CITES II rules of endangered species. To people and scientists of the village of Taiji, Japan, and the whale museum there, especially Mr. Yoji Kita, and to Dr. Wered Werner, who personally brought the ears back from Japan to Jerusalem. Dr. Darlene Ketten PhD, at Woods Hole Oceanographic institute in Cape Cod, Massachusetts, USA, has been so helpful in working out and interpreting the CT scans of the ears, and offered very helpful remarks during the following years. Dr. Cristina Lockyer, at the time a visiting scientist in the Ministry of Fisheries, Copenhagen, Denmark, aged the whales from sections of their teeth. I am grateful to Professor Jim Saunders, who made the contact with and asked Professor John Rosowski to receive me in his lab, and Professor Rosowski, Eaton-Peabody Laboratories in the Ear and Eye Infirmary of Harvard Medical School, Boston USA, who, for a whole week, carried out the Laser Vibrometry experiments for the first paper of the thesis. Professor Nir Shaviv, Physics department of the HUJ, interpreted our findings and carried out the computations relating to the results of paper one, as well as the modelling of the results of the asymmetry paper. I am indebted to Dr. Yael Seligman for performing all the statistical analyses in all three papers with great diligence and care to Dr. Boaz Shacham for helping in the design of many of the tables and graphs appearing throughout, and to Mor Tsur, Akiva Topfer and Mrs. Nurit Werner for their help in the illustrations. Professor Ran Nathan, of the Department of Ecology, Evolution and Behavior, HUJ, has generously given me the use of his lab for carrying out all the weighing of all the ossicles, those of *Globicephala*, as well as those of the cat and sheep.

Mr. Zayd Abu Gharbiya, from Bethlehem, Palestine, helped me both to obtain and split the sheep skulls, a process in which we were both excluded from a few butchers' shops in Bethlehem, after having destroyed their band saws in the process…Zayd and I are a living proof that Palestinians and Israelis can not only live in Peace, but have created a long-lasting, respectful and loving relation between our two families. This is a "side branch" aspect of this thesis, which we both are very proud of: Thank you Zaid, Wanda, Osama, Ibrahim, Zainab, Fatima and sweet little Omar, for showing the world that Peace between us is not only possible, but is indeed the only way forward.

My Surgery teacher (soft tissues) and lifelong mentor, Professor Bruce Christie, Beechworth Australia, has carried out the amazing, top quality dissections and computer pictures of the sheep and cats ossicles, with the help of the Anatomy Department of Charles Sturt University, Wagga Wagga, Australia. He has been my constant inspiration both in and outside the operating theatre, combining the love of operating with the eternal love of God, the "Master Engineer", the creator of "All creatures, great and small, the Lord God has made them all". In this connection, I am indebted to my veterinary orthopedics teacher, Dr. Hamish Denny FRCVS, who, during the years 1984-1985, in Bristol University Vet School, inspired me to adopt my lifelong profession as a veterinary orthopedic surgeon, which enabled me to fund all the research carried out in this long and wonderful journey.

I am most grateful to Dr. Maria Morell PhD, the University of Veterinary Medicine, Hanover, Germany and Associate Professor Magnus Wahlberg PhD, University of Southern Denmark, Odense, Denmark, for acting as pre-examiners, critically reading the thesis, offering their valuable remarks and recommending it to be presented for public examination as a doctoral dissertation at the University of Helsinki; and of course to Professor Peter Tyack PhD, St. Andrews University, Scotland, for accepting the role of the Opponent on the 24th September 2020, albeit done by "Zoom" from a distance, Corona virus dictating.

And of course none of this would have materialized if it were not for the *unearthly kind and knowledgeable academic triumvirate* in the Faculty of Biological and Environmental Sciences, Molecular and Integrative Biosciences Research Program, University of Helsinki. Professor Juha Voipio, Head of Department, has created a framework in which I, as a total stranger, could fully integrate to the department and meet all the formal and academic requirements needed to complete the degree. How often has anyone met a Head of Department, inundated with the most pressing academic and administrative obligations, *physically* taking my student's registration papers to the registry office, sorting out all the formalities for me, in order to save me spending time "in the zone of administrative ambiguities" where one finds oneself, esp. with the physical distance separating Jerusalem from Helsinki? I would also like to thank Professor Tomi Taira PhD, who joined him as a co-member of my internal Helsinki committee, for taking on this task.

My academic supervisor, Professor Kristian Donner, has made the journey so smooth, covering bitter pills of necessary criticism in such a way that they were so easy to swallow and accept. Together with Dr. Nummela, he edited all the aspects of the thesis, as well as taking it up the ladder of all the formal stages, including the internal Helsinki committee, choosing the external referees and finally my opponent. To say nothing of our continuous PHILOSOPHICAL discussions, spanning from Aristotle in our first meeting, to classical music, Finnish, Nordic and Jewish cultures (Notably the Golem and Schtisel, to which he introduced me…), linguistic origin of scientific and popular terms, all given to me in the most caring and loving way.

Last but definitely not least: Dr. Sirpa Nummela PhD, whose doctoral thesis from 1999 and all her publications in the following years, served as the basis for this thesis; I hope to have added some relevant and new information to her seminal work in this area. Dear Sirpa: You have been the *Florence Nightingale* of my thesis, nursing all the injuries I have inflicted upon it in places, going

around with the oil lamp to its darker corners, looking to change the dressings where needed, and giving me all of your encouragement, support, and tender loving care, based on your phenomenal knowledge. I was stunned by your amazing dissecting skills and patience, when you came to Israel to harvest those tricky sheep ossicles. In the ancient temple which stood in Jerusalem there were the Great Cohanim (Jewish religious leaders), who carried out the holy work of the temple. You are and have been the Great Cohenet (הכוהנת הגדולה) in the temple of Cetacean knowledge, to which I hopefully have added my very modest offerings. In this context, I should also like to thank your scientific mentors and collaborators Dr. Simo Hemilä and Professor Tom Reuter, for their kind interest and valuable suggestions offered when I first approached the University of Helsinki.

I warmly recommend to all of you reading the story "The Door in the Wall" by H.G. Wells. It tells about a boy whose mother died at birth, and who grew up distant from his stern father; one day he went through a mysterious green door in a white wall in Kensington, to find himself in an enchanted garden, the magic of which has haunted him for the rest of his life. The University of Helsinki has been my very magical garden, and the time I have spent with all three of you, Sirpa, Kristian and Juha, in the last eight years, has been nothing short of a spiritual experience of the highest quality.

I shall be indebted to you for the rest of my life for the part you had in achieving my biggest dream. I hope this thesis will find a place next to yours, Sirpa, on the shelves of the University of Helsinki.

Itamar Tsur, DVM
Helsinki and Jerusalem, September 2020.